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Microbial Ureases: Significance, Regulation, and Molecular Characterization†

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INTRODUCTION

Enormous quantities of urea ($H_2N—\overset{\overset{O}{\|}}{C}—NH_2$) are constantly released into the environment through biological actions. For example, urea is excreted in the urine of all mammals as a detoxification product (237). Human urine contains 0.4 to 0.5 M urea (76), which results in an annual release of 10 kg of urea per adult (237). Birds, reptiles, and most terrestrial insects excrete uric acid as their primary detoxification product; however, environmental degradation of this compound also yields urea. Urea also is a well-known product of general purine catabolism (238), and urea release accompanies the biodegradation of nitrogenous compounds such as arginine, agmatine, allantoin, and allantoic acid (238). Urea generated by these reactions is generally short-lived because of further metabolism involving the enzyme urease.

Urease (urea amidohydrolase; EC 3.5.1.5) catalyzes the hydrolysis of urea to yield ammonia and carbamate, which spontaneously hydrolyzes to form carbonic acid and a second molecule of ammonia (1).

$$H_2N—\overset{\overset{O}{\|}}{C}—NH_2 + H_2O \xrightarrow{\text{urease}} NH_3 + H_2N—\overset{\overset{O}{\|}}{C}—OH$$

$$H_2N—\overset{\overset{O}{\|}}{C}—OH + H_2O \longrightarrow NH_3 + H_2CO_3$$

At physiological pH, the carbonic acid proton dissociates and the ammonia molecules equilibrate with water to become protonated, resulting in a net increase in pH.

$$H_2CO_3 \rightarrow H^+ + HCO_3^-$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^-$$

The best-studied urease is that from jack bean (1, 14). This plant urease was the first enzyme to be crystallized (220) and also the first shown to contain nickel (46). Many eucaryotes synthesize urease, including plants other than jack bean (68, 181), some invertebrates (148), and numerous eucaryotic microorganisms (described below). Also, urease activity is widely observed among the procaryotes, including many eubacteria (discussed below) and at least one archaebacterium, *Methanobacterium thermoautotrophicum* (12). By 1947, more than 200 ureolytic microorganisms encompassing a wide range of divisions had already been reported (221). However, within a division, some representatives have urease and some do not. Indeed, because of the ease of assay, ureolysis is often a criterion for identification of certain genera and species (17, 120). For example, *Ureaplasma* strains are distinguished from other mycoplasmas by their ability to hydrolyze urea (209). Similarly, the high levels of urease activity in *Proteus* strains are used to distinguish them from many other *Enterobacteriaceae* family members (36).

A distinct mechanism for urea degradation involving urea amidolyase is present in many yeasts and in chlorophyceaen algae (9, 124, 192). This adenosine triphosphate (ATP)- and biotin-dependent enzyme system (191) is actually composed of urea carboxylase and allophanate hydrolase activities and will not be further discussed here.

This review will summarize the medical and ecological significance of microbial ureases, the regulation and cellular localization of the protein, the physical and kinetic characteristics of the enzyme, and the organization and functions of microbial urease genes. When appropriate, the microbial ureases will be compared and contrasted to the well-characterized plant enzyme.

SIGNIFICANCE OF MICROBIAL UREASES

Microbial ureases are important enzymes in certain human and animal pathogenic states, in ruminant metabolism, and in environmental transformations of certain nitrogenous

compounds. The role of urease in each of these contexts will be discussed.

Role of Urease in Pathogenesis

Bacterial urease is implicated in the pathogenesis of many clinical conditions. It is directly associated with the formation of infection stones and contributes to the pathogenesis of pyelonephritis, ammonia encephalopathy, hepatic coma, urinary catheter encrustation, and peptic ulceration. It has been suggested that urease may have a role in the inactivation of complement as well.

Infection stones. Infection-induced stones, which account for 15 to 20% of all urinary stones (71, 77, 194), are a mixture of struvite ($MgNH_4PO_4 \cdot 6H_2O$) and carbonate apatite [$Ca_{10}(PO_4)_6 \cdot CO_3$]. The normally soluble polyvalent ions become supersaturated as the pH increases from 6.5 to 9.0, which occurs when ammonia is released by microbial urease-catalyzed urea hydrolysis. This alkalinization results in the stone crystallization.

In humans, *Proteus mirabilis* is the most common organism implicated in stone formation (193). Other urease-producing species associated with infection stones include *Pseudomonas*, *Klebsiella*, and *Staphylococcus* spp. (71); *Corynebacterium* sp. group D2 (213); and *Ureaplasma urealyticum* (84). Recently, the first description of urinary calculi by *Proteus penneri*, a newly classified species, was reported by Krajden et al. (118). In addition, *Providencia stuartii*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Morganella morganii* are often associated with spinal injury patients whose kidney stones are related to persistent polymicrobial bacteriuria caused by long-term catheterization (194, 241, 242). *Escherichia coli*, generally urease negative, is rarely implicated in stone formation (71).

Several urease-producing organisms have been reported to cause struvite stones in certain animals. For example, *Staphylococcus saprophyticus* is associated with stone formation in minks (173); *Staphylococcus aureus*, in schnauzer dogs (109); and possibly *U. urealyticum*, in cats (149). Furthermore, Nguyen et al. (170) isolated *S. aureus* from a ferret stone.

In addition to microbial ureolytic activity, it is speculated that organic substances contribute to formation of the infection stone matrix. Griffith (72) suggested that host mucoproteins from the urothelial surface may serve as a nidus for stone formation. Bacterial glycocalyx may also play an important role in infection stone maturation (150, 171, 172, 216). Morphological examination of struvite calculi indicates that bacteria are present throughout the stone (150, 226). Microscopic studies of struvite stones removed surgically from patients after antibiotic treatment demonstrated that bacteria are present in glycocalyx-enclosed microcolonies on the surface, in stone interstices, and within the core of these calculi (171). McLean et al. (150) and Nickel et al. (171) proposed that the glycocalyx from infecting bacteria forms an initial surface for adherence; urease activity then causes struvite and apatite precipitation and entraps the resultant crystals as well as host mucoprotein. Grenabo and others (70) demonstrated that infection with *Proteus mirabilis*, *E. coli*, enterococci, and *Ureaplasma* spp. predisposed the rat bladder to adherence of urease-induced crystals produced in vitro. The bacteria within this matrix are protected from antibiotic action, resulting in a persistent infection, further stone growth, and potentially severe renal damage.

Griffith et al. (76) conducted in vitro crystallization experiments to study the role of bacterial urease in stone formation. Inoculation of human or synthetic urine with *Proteus* or jack bean urease resulted in the development of alkalinity, supersaturation, and consequent precipitation and crystal encrustation on glass rods. Crystallization could be prevented by the addition of acetohydroxamic acid, a urease inhibitor. Other organisms that rarely produce urease, such as *E. coli*, or synthesize low levels of urease, such as *Klebsiella* or *Pseudomonas* spp., did not induce significant sediment accumulation or rod encrustation. The urine pH did not change in the presence of *E. coli* and only slightly changed in the presence of urease-positive *Klebsiella* or *Pseudomonas* spp. Similarly, Stegmayr and Stegmayr (216) demonstrated a loss of soluble Ca^{2+} and Mg^{2+} ions (i.e., precipitation) in urine inoculated with *Proteus* strains. Alkaline pH alone was not sufficient to cause a significant drop in the level of soluble divalent cation, but after *Proteus* inoculation a 50-fold drop in soluble Ca^{2+} levels was measured (216). Inoculation with killed *Proteus* sp., however, did not prompt precipitation.

Urease-induced stones commonly manifest as matrix concretions in the renal pelvis, branched or staghorn renal calculi, or bladder calculi (72). Management strategy involves surgical removal or the use of high-energy shock waves to break up kidney stones in situ (lithotripsy), as well as administration of antimicrobial agents (193). Urease inhibitors such as acetohydroxamic acid or hydroxyurea have been shown to be beneficial postoperative chemotherapeutic agents (71, 142). In the absence of surgery, clinical trials (31, 73–75, 244) demonstrated that oral administration of acetohydroxamic acid or hydroxyurea lowered urinary pH of bacteriuric patients and reduced the size of urinary stones. However, half or more of the patients experienced side effects that required stopping treatment or reducing the size of the dose. As complications, acetohydroxamic acid inhibits complement activity (211) and can cause reversible hemolytic anemia and thrombophlebitis. Furthermore, both agents inhibit deoxyribonucleic acid (DNA) synthesis, depress bone marrow biosynthesis, and are teratogenic in high doses (72).

Pyelonephritis. Pyelonephritis is defined as an acute or chronic inflammation of the kidney and its pelvis. Whereas chronic pyelonephritis may or may not involve bacterial infection (197), acute pyelonephritis results from bacterial infection and is characterized by interstitial inflammation and tubule necrosis. Although *E. coli*, a primarily nonureolytic species, causes a majority of cases of human acute pyelonephritis (197), urease elaborated by other infecting species appears to contribute significantly to tissue damage, inflammation, and cell invasion.

Proteus mirabilis is the primary urease-producing uropathogen in humans (197). The role of *Proteus* urease in pyelonephritis was studied by Braude and Siemienski (20) by using both a rat model and a tissue culture system and by MacLaren (131), Gorrill (67), and Fitzpatrick (55) by using mouse models. In each case, pyelonephritis was induced by *Proteus* infection. The direct toxicity of urease on renal tissue was demonstrated by using killed *Proteus* suspensions with active and inactivated enzyme; necrosis occurred only with the active enzyme (20). Intracellular tissue culture infection with *Proteus mirabilis* increased as urea concentration rose (20). In the mouse model, an ethyl methanesulfonate-generated urease-negative mutant of *Proteus mirabilis* produced much smaller renal abscesses than the parent strain and lower populations in the kidney (131).

In contrast to *Proteus* spp., inoculation with *E. coli* produced almost no intracellular infection in kidney cells, no

rise in pH, and no kidney cell injury in tissue culture (20). Along these lines, Parsons et al. (175) demonstrated that exposure of rabbit bladders to ammonium ions in vivo increased bacterial adherence to transitional epithelium.

Others examined the specific effects of urease deficiency in animal models of pyelonephritis by treating animals infected with *Proteus mirabilis* with acetohydroxamic acid (3, 132, 166). In all studies, kidney abscesses were smaller or absent in the acetohydroxamic acid group as compared with animals receiving no inhibitors. In addition, treated animals yielded fewer organisms and suffered few deaths.

Bovine pyelonephritis is caused by *Corynebacterium renale* (94), an organism that possesses an active urease. Infection with this microorganism resulted in alkaline urine, growth of *Corynebacterium renale* from kidney homogenates and urine, and necrosis of the renal tissue (94). Subsequently, Jerusik et al. (93) showed that in experimentally infected rats the use of acetohydroxamic acid at high doses reduced the urine pH, decreased the number of colony-forming units of *Corynebacterium renale* in the kidney, and halted necrosis of the kidney tissue. Infection with a urease-negative mutant of *Corynebacterium renale* did not result in pyelonephritis. However, the rats developed cystitis. This evidence suggests that the ammonia liberated from ureolysis causes the alkalinization of the urine and may account, in part, for the necrosis of kidney tissue associated with pyelonephritis (93, 94).

Ammonia encephalopathy, hepatic encephalopathy, and hepatic coma. Urease activity has been implicated in contributing to hepatic encephalopathy (202, 210) and coma (200), both of which are manifestations of portal-systemic encephalopathy. Hepatic coma occurs when the brain becomes intoxicated by nitrogenous compounds originating from intestinal sources that have not been metabolized by the liver (210). Ureolytic organisms contribute to hepatic encephalopathy and coma by the production of ammonia. Although the role of ammonia metabolism in the pathogenesis of portal-systemic encephalopathy is not entirely understood, therapeutic measures to reduce the numbers of urea-hydrolyzing organisms have proven effective (200). Hyperammonemia was also shown to be reduced by administration of acetohydroxamic acid (53, 219).

Inactivation of complement by urease. Host defense mechanisms against enteric bacteria are compromised in the kidney tissue by inactivation of complement. Beeson and Rowley (8) reported that kidney tissue inactivated the fourth component of complement and attributed this to the release of ammonia by the action of renal glutaminase. A parallel was demonstrated between the quantity of ammonia liberated and the extent of anticomplementary effect. The role of renal glutaminase in this effect was supported by the observation that anticomplementary activity was enhanced by glutamine. In light of the fact that kidney tissue is also rich in urea, MacLaren (131) suggested that ammonia generated by urease-producing organisms probably also contributes to this anticomplementary effect.

Urinary catheter obstruction. The catheterized urinary tract appears to offer an excellent niche for colonization by urease-producing organisms such as *Proteus mirabilis*, *Proteus vulgaris*, *Providencia stuartii*, *Morganella morganii*, and *Providencia rettgeri* (160, 242). The importance of urease-producing organisms in catheter encrustation, which may lead to obstruction, has been demonstrated by several groups. For example, Norberg et al. (174) isolated *Proteus* strains from 13 of 15 patients with catheters removed because of obstruction; however, the frequency of *Proteus* isolation was not reported for catheters removed for other reasons. Burns and Gauthier (29) demonstrated that catheter encrustation in five patients with long-term indwelling catheters could be significantly reduced by the administration of acetohydroxamic acid. To identify the species implicated in obstruction of chronically catheterized patients, a prospective study was carried out by Mobley and Warren (160). Although 86% of urine specimens yielded at least one urease-positive species, *Proteus mirabilis* was the only species significantly associated with obstruction. Bruce et al. (26) found that samples of catheter encrustation were composed of calcium, phosphorus, and magnesium, similar to the composition of infection stones.

Peptic ulceration. The recently described species *Campylobacter pylori* (formerly *C. pyloridis*) produces a potent urease and has been implicated in the development of peptic ulceration (66, 83, 157). Several lines of investigation suggest that *C. pylori* infection is an important etiological factor in the development of upper gastrointestinal inflammatory lesions. Ingestion of *C. pylori* by two volunteers was followed by development of acute dyspeptic syndromes associated with histological evidence of gastritis (141, 162). Other studies have demonstrated resolution of gastritis subsequent to (or following) antimicrobial therapy which eradicated *C. pylori* infection (64, 153, 231). More recently, *C. pylori* infection of the stomach of the neonatal gnotobiotic piglet reproduced many of the features of the disease in humans (119, 122).

C. pylori is acid sensitive and replicates in a pH range of 6.9 to 8.0 (66), yet it appears to be uniquely adapted for survival in the highly acidic environment of the human stomach. This microorganism produces high levels of urease activity. It has been postulated that urease activity may be an important survival factor for *C. pylori* by generating a "cloud" of ammonia which would protect the bacterium from stomach acid (19). A number of postulated mechanisms have been proposed by which urease may also represent a virulence factor for *C. pylori* and contribute to gastric mucosal damage. First, urea hydrolysis could increase the mucosal surface pH, preventing normal passage of hydrogen ions from gastric glands to gastric lumen and permitting back-diffusion of hydrogen ions (83). Second, high NH_3 concentrations could be directly toxic to intercellular tight junctions and alter gastric mucosal permeability (33, 83). It is highly probable that urease is critical for colonization of the stomach by *C. pylori* and may contribute significantly to the pathogenicity of this organism.

Urease produced by strains of *C. pylori* is biochemically and genetically distinct from ureases produced by species common to the urinary tract such as *Proteus*, *Providencia*, and *Morganella* (51, 157). With a K_m for urea of 0.8 mM, urease of *C. pylori* binds substrate with much higher affinity than ureases produced by these latter bacterial species (157). This observation is physiologically consistent with the biologic niches of the respective organisms: *C. pylori*, adjacent to gastric mucosa, must scavenge urea from serum at physiological blood urea concentrations of 1.7 to 3.4 mM, whereas uropathogens are exposed to saturating urea concentrations in urine.

Urease in the Rumen and Gastrointestinal Tract

Microbial ureases play an important role in the nitrogen metabolism of ruminants such as cattle, sheep, and other animals that contain a forestomach (87). Substantial amounts of animal-derived urea are recycled to the rumen, where

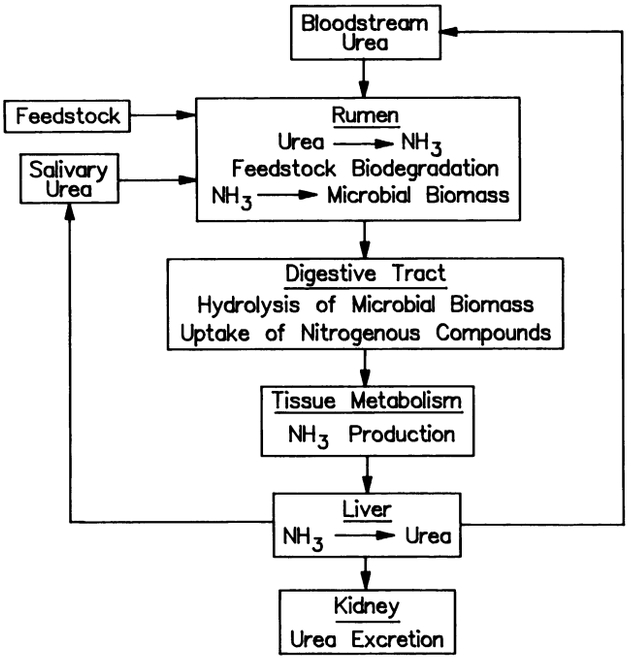

FIG. 1. Nitrogen cycling system in ruminants. A significant proportion of the nitrogen requirement of ruminal microorganisms is met by urea, which diffuses into the rumen from the bloodstream or enters with saliva. Urea is hydrolyzed by microbial ureases, and the product ammonia is taken up by the ruminal microorganisms. Microbial biomass is subsequently degraded by the ruminant digestive system to supply nitrogen-containing compounds to the animal. Tissue metabolism releases ammonia, which is transformed into urea by the liver.

ureolytic activity releases ammonia, the major source of nitrogen for most ruminal bacteria (27). The microbial biomass generated is then utilized as a nutrient by the ruminant. The concept of a ruminant nitrogen cycle is illustrated in Fig. 1. Exogenous urea can be used to supplement nitrogen-deficient feedstocks to enhance the quality of the ruminant diet. Urea hydrolysis also occurs in the intestinal tract of monogastric species such as humans, pigs, rats, cats, mice, and rabbits (237), but nitrogen cycling is quantitatively less important in these organisms compared with ruminants.

Sheep ruminal urease. Tracer studies have demonstrated that endogenous urea is supplied to the rumen by direct transfer from the bloodstream and by preliminary transfer into saliva which is then swallowed (105). Up to 25% of the nitrogen used by ruminal microorganisms can be supplied by urea recycling (149). By using gnotobiotic lambs (containing a defined microbial population), urea transfer to the rumen was shown to occur by simple diffusion; however, the presence of ureolytic bacteria attached to the ruminal epithelium accelerated the rate of urea flux by maintaining a lower urea concentration in the rumen (34). This process is somewhat self-regulated as the ruminal urease activity was repressed by ammonia (34).

Ureolytic microorganisms in sheep rumen are limited to the bacteria, 35% of which were estimated to possess urease activity (99). The identity of the major ureolytic species is not clear. In one study (233), *Staphylococcus saprophyticus* and *Micrococcus varians* were shown to possess urease. In a separate study (39), ureolytic species of *Staphylococcus*, *Lactobacillus casei*, and *Klebsiella aerogenes* were identified. By enrichment culture a ureolytic strain of *Streptococcus faecium* was also isolated (39). Both research groups described the apparent loss of urease activity when several ureolytic ruminal strains were subcultured (40, 233). In neither study was an obligately anaerobic urease-producing strain observed. This negative result may be due to the failure of the urease tests in use to identify ureolytic microorganisms because the growth medium leads to repression of urease activity (234).

Cattle ruminal urease. Nitrogen cycling in the bovine rumen (177) is similar to that observed in sheep, and the ureolytic microorganisms participating in this environment are well characterized. Using a minimal medium to prevent urease repression, Wozny et al. (246) identified ureolytic strains of *Succinovibrio dextrinosolvens*, *Treponema* sp., *Ruminococcus bromii*, *Butyrivibrio* sp., *Bifidobacterium* sp., *Bacteroides ruminocola*, and *Peptostreptococcus productus*. In addition, a well-studied ureolytic strain of *Selenomonas ruminantium* (212) was isolated by using roll tube medium with urea as the main nitrogen source (96). In contrast to the studies of sheep rumen, most of the ureolytic bacteria in cattle rumen are strict anaerobes.

Human intestinal urease. In humans, approximately 20% of the urea produced by the liver is transferred by diffusion from the bloodstream to the intestinal tract and hydrolyzed by urease (237). As discussed above, several pathological states are associated with excessive intestinal urea hydrolysis. U.S. investigators have reported that the most numerous ureolytic bacterium in human feces was *Peptostreptococcus productus* (234), although ureolytic strains of *Ruminococcus albus*, *Clostridium innocuum*, *Clostridium beijerinckii*, *Fusobacterium prausnitzii*, *Coprococcus catus*, and *Streptococcus mitis* were also isolated (246). Japanese investigators have found that the predominant ureolytic strains included *Eubacterium aerofaciens*, *Eubacterium lentum*, and *Peptostreptococcus productus*, with ureolytic strains of *Bacteroides multiacidus*, *Bacteroides bifidum*, *Clostridium symbiosum*, *Fusobacterium necrophorum*, *Fusobacterium varium*, *Lactobacillus fermentum*, *Peptococcus asaccharolyticus*, and *Peptococcus prevotii* also observed (222). It is unclear whether the differences observed in these two studies may be attributed to geographical or human population distinctions. As in the case of the bovine rumen, most ureolytic bacteria in the human intestine are anaerobes.

Environmental Urease

Urease activity is widely distributed in soil and aquatic environments, where it plays an essential role in nitrogen metabolism (25). For example, degradative processes involving protein and nucleotide turnover require urease activity. More importantly, effective urea fertilization requires controlled ureolysis to enhance efficiency and minimize crop damage.

Importance. The availability of fixed nitrogen is the major limitation to agricultural productivity. Two major forms of fixed nitrogen in the environment are proteins and polynucleotides. These components are hydrolyzed to the amino acid and nucleotide monomers and then further degraded by a variety of enzymes. The guanidino group of arginine and the ring nitrogens of purines are released as urea, which is further hydrolyzed by urease (235, 238). The product ammonia can be taken up and utilized by soil microbes and plants. However, in many environments the level of available nitrogen compounds is inadequate for optimal crop production. Therefore, fertilizers are applied which can be converted to a form of nitrogen that plants can assimilate. Urea

is widely used because of its low cost, ease in handling, and high nitrogen content (7). To emphasize the scale of urea usage, chemical production of urea in the United States was 14.86 billion pounds (ca. 6.74 billion kilograms) in 1987 (187), most of which was used in fertilizers.

Urea fertilization does pose an inherent problem, however. Uncontrolled urea hydrolysis can lead to plant damage from ammonia toxicity or elevated pH. Furthermore, low efficiency of urea fertilizer utilization (e.g., loss of 50% of applied nitrogen) is observed in basic soils, because volatile ammonia escapes into the atmosphere (164, 201). Similarly, only 25 to 35% of applied nitrogen is recovered by submerged crops such as rice (30). Several groups have examined the potential for combining urease inhibitors with fertilizer urea to reduce the rate of ureolysis, minimize crop damage, and enhance nitrogen utilization efficiency. Early studies indicated that dihydric phenols and quinones were effective in decreasing soil urease activity (23). In addition, a series of hydroxamic acids, which were known to be specific urease inhibitors (112), were shown to reduce the rate of ammonia loss from soil urea (183). In recent laboratory and greenhouse experiments, a number of phosphoryl amides and thiophosphoryl amides were also shown to retard urea hydrolysis by inhibiting urease (22, 30, 125, 143, 185). Whereas urea fertilization typically has adverse effects on seed germination, seedling growth, and early plant growth, inclusion of these inhibitors in urea fertilizer eliminated such problems in alfalfa, barley, oats, rye, sorghum, and wheat (24). However, in field evaluations of the urease inhibitor phenylphosphorodiamidate, no increase in the yield of corn was detected (228). Nevertheless, the potential value of this approach is an incentive to examine new urease inhibitors (203).

Urease activity in the environment. Microbial urease activity is widespread in the environment and includes the action of bacteria, yeasts, filamentous fungi, and algae (9, 11, 17, 90, 91, 100, 188, 205, 221, 235). It is not the purpose of this section to provide a comprehensive list of ureolytic microorganisms, but rather to emphasize the broad distribution of this activity and to comment on possible alternative functions of urease in some of these microbes.

In a sampling of six different soils, 17 to 30% of the cultivable bacterial population was ureolytic (127). However, much of the urease activity in soil is also due to extracellular enzyme (164). This cell-free enzyme, probably derived from plant and microbial cells, is remarkably stable presumably because of interaction with soil components (18, 164). Comparison of the cell-associated and cell-free forms of the enzyme is essential for evaluating the effectiveness of urease inhibitors to be used in urea fertilizers.

Some soils possess only low levels of urease and may be unable to utilize fertilizer urea fully. Low urease activity may be due to depressed environmental nickel levels, since all ureases examined are nickel-containing enzymes (81). Stimulation of urease activity in nickel-depleted soil by addition of nickel ion has been examined (43). Slight enhancements in urease activity were observed; however, no improvements in crop yield were noted.

The high cellular content of urease in some microbes suggests that this protein may have functions in addition to catalytic activity. For example, urease makes up 1% of the cell dry weight of *Bacillus pasteurii*, a common soil bacterium (123), and 8.5% of the total soluble protein of the fungus *Aspergillus tamarii* (248). In *Aspergillus tamarii*, the enzyme was suggested to also function as a storage protein (248). Studies should be conducted with *Aspergillus tamarii*, *Bacillus pasteurii*, and other microorganisms to examine the turnover rates of urease, in comparison to other proteins, when cells are starved or shifted to different growth media.

PHYSIOLOGY OF MICROBIAL UREASES

Urease Regulation

Ammonia, a product of urea hydrolysis, is the preferred nitrogen source among enteric bacteria (230) and can be assimilated into a variety of nitrogenous compounds via glutamine. Glutamine is synthesized by addition of ammonia and glutamate, a reaction catalyzed by glutamine synthetase. It was earlier hypothesized that synthesis of many enzymes related to nitrogen metabolism, including urease (59), was regulated by glutamine synthetase (89, 135). However, this proposal was later found to be incorrect as the nitrogen regulation system was shown to be governed by a complex cascade that ultimately triggers synthesis of a ribonucleic acid (RNA) polymerase that recognizes specific promoters of nitrogen-regulated gene products (for a recent review, see reference 136). In many bacterial species, the production of urease appears to be tightly regulated in conjunction with this nitrogen regulatory system. For example, urease synthesis can be repressed in the presence of ammonia- or nitrogen-rich compounds (including urea) that release ammonia; synthesis is derepressed under nitrogen-limiting or nitrogen starvation conditions. Other bacterial species appear to respond directly to substrate urea as an inducing agent. A third class of ureases appears to be produced constitutively, and synthesis is not affected by addition or limitation of ammonia, urea, or other nitrogenous compounds.

Another aspect of urease regulation involves possible changes in enzyme levels at different stages of cellular differentiation. Using *Proteus mirabilis* and *Proteus vulgaris*, Falkinham and Hoffman (48) reported dramatic differences in urease activities for the elongated swarm cell (the differentiated cell type which gives rise to the well-known "swarming" phenomenon on agar plates) as opposed to the typical short unit cell. The highly flagellated swarm cells were reported to be constitutively ureolytic, whereas nonflagellated short cells had negligible urease levels and were noninducible (48). These intriguing results were surprising when compared with broth culture studies in which short cells predominate; liquid cultures were known to possess high levels of inducible urease (195, 196). Recently, Jin and Murray (95) demonstrated that both short and swarmer cells produced urease in an inducible manner, at odds with the earlier work. The differences reported in these studies may be attributed to strain selection, experimental methods, or other changes. It may be worthwhile to test for cell differentiation-dependent urease regulation in other species such as *Bacillus* during sporulation and *Azotobacter* during encystment.

Table 1 lists bacterial and fungal species for which the type of regulation has been investigated. Methods for determining urease activities as well as the definitions of constitutive, inducible, and repressible varied, and results were interpreted to the best of our ability.

Cellular Localization of Urease

Although urease is occasionally described as an extracellular enzyme (17, 188), and some studies indicate a periplasmic or membrane-bound location (see below), considerable

TABLE 1. Regulation of urease production

Organism	Mechanism of urease regulation[a]			Reference(s)
	Constitutive	Inducible	Repressible	
Agrobacterium tumefaciens	+			45
Alcaligenes eutrophus			+	100, 116, 117, 120
Anabaena cylindrica	+			130
Anabaena doliolum			+	184
Anacystis nidulans			+	184
Arthrobacter oxydans			+	204
Aspergillus nidulans		−	+	129
Bacillus megaterium			+	100
Campylobacter pylori	+	−		51, 157
Chromatium vinosum	+			5
Corynebacterium renale	+			126
Klebsiella aerogenes		−	+	59, 103; Mulrooney and Hausinger, submitted
Micrococcus cerificans			+	100, 120
Morganella morganii	+			196, 206
Neurospora crassa	+			82
Paracoccus denitrificans			+	110, 120
Peptostreptococcus productus			+	234
Proteus mirabilis		+		95, 98, 195, 206
	+	−		48
Proteus penneri		+		159
Proteus vulgaris		+		195
	+			48
	+		−	100, 120
Providencia rettgeri		+	+	133
		+		195
			+	249
Providencia stuartii		+		158
Pseudomonas acidovorans		+	+	100
Pseudomonas aeruginosa			+	89, 100
Pseudomonas fluorescens			+	100
Rhizobium leguminosarum	+			92
Selenomonas ruminantium		+	+	96
			+	212
Sporosarcina ureae	+			120
			−	100
Thiocapsa roseopersicina		+	+	5
Thiocystis violacea	+			5

[a] +, Type of regulation observed; −, type of regulation determined not to occur.

evidence indicates that the enzyme is cytoplasmic in both yeasts and bacteria. Jeffries (90, 91) demonstrated that urease activity in 22 species of bacteria was associated with soluble extracts of the cells, which were ruptured by sonication or French pressure cell lysis. In cell fractionation studies of *K. aerogenes* (59), *U. urealyticum* (44, 145, 189, 236), *Providencia stuartii* (158), and *Proteus mirabilis* (98), the majority of urease partitioned with the soluble cytoplasmic fraction. Analysis for control enzymes, known to reside in specific cell fractions, supported the conclusion that urease is cytoplasmic in these species.

Contrary evidence obtained by electron microscopic methods suggested that *Staphylococcus* urease was membrane bound (151) and that *Proteus mirabilis* urease was associated with the periplasm and outer membrane (152). In these experiments, bacteria were incubated with sodium tetraphenylboron in the presence and absence of urea, the ammonia-reagent complex was precipitated, silver ions were exchanged for precipitated ammonia, thin sections were prepared, and electron-dense material was visualized. It should be noted that the reagent reacts with a product of urea hydrolysis and not the enzyme itself. In addition, it was not demonstrated that the bacterium was permeable to tetraphenylboron. Cell fractionation supported the morphological observations in the *Proteus mirabilis* study (152), but partitioning of control enzymes was not reported.

Vinther (236) studied urease localization in *U. urealyticum* also by electron microscopy methods based on precipitation of electron-dense MnO_2 which is insoluble at alkaline pH. Cells, incubated in the presence of both urea and Mn^{2+}, were thin sectioned and examined by transmission electron microscopy, which revealed heavy deposits of MnO_2 in the cytoplasm that were not associated with the membrane, an observation consistent with fractionation studies.

For comparison with the above microbiological studies, jack bean seed urease was also shown to be cytoplasmic by immunocytochemical and biochemical methods (50).

Ureaplasma Energy Transduction

Urea hydrolysis may play a novel role in *U. urealyticum* by functioning in energy transduction. Ford and MacDonald (57), and later others (56, 60, 107, 144), demonstrated that urea was an essential substrate and that urease was an essential enzyme for growth of *Ureaplasma* spp. However, most of the ammonia generated by urea hydrolysis was released into the medium; very little was assimilated by the cells. The same was true of [^{14}C]-urea carbon, which was liberated mainly as $^{14}CO_2$ (58). Therefore, in this organism neither product of urea hydrolysis was used at significant levels by the cell. Masover et al. (145) reasoned that ammonia generated by urease would be protonated at physiological pH, resulting in ammonium ion (NH_4^+) formation. This ion, unlike freely permeable NH_3, should diffuse only slowly across the membrane, resulting in an ion gradient and membrane potential that would drive ATP synthesis via a membrane bound adenosine triphosphate synthase. Romano et al. (190) provided experimental data to buttress this hypothesis. Urea added to resting *Ureaplasma* cells resulted in a rapid increase in intracellular ATP concentrations, suggesting that proton motive force was involved. Further work with ionophores suggested that the pH gradient (ΔpH) generated by urea hydrolysis and not the electrical potential ($\Delta\psi$) drove ATP synthesis. Acetohydroxamic acid, a urease inhibitor, diminished ATP synthesis following addition of urea. Flurofamide, another potent urease inhibitor, was later used to demonstrate specific growth inhibition of the *Ureaplasma* spp., but not of the closely related *Mycoplasma* sp. (61, 106). A limitation of the *Ureaplasma* energy transduction hypothesis is that only transient ATP synthesis was experimentally observed. It is unclear how the cell could dispose of intracellular ammonium ion to allow continuous, urease-dependent ATP synthesis. A model for urease-coupled energy transduction has been incorporated into Fig. 2, which depicts transport processes relevant to ureolysis by bacterial cells.

Urea and Ammonium Ion Transport

Measurement of whole-cell urease activity involves four processes: entry of urea into the cell, hydrolysis, exit of ammonia, and quantitation of the released ammonia or measurement of the pH increase. Membranes are often

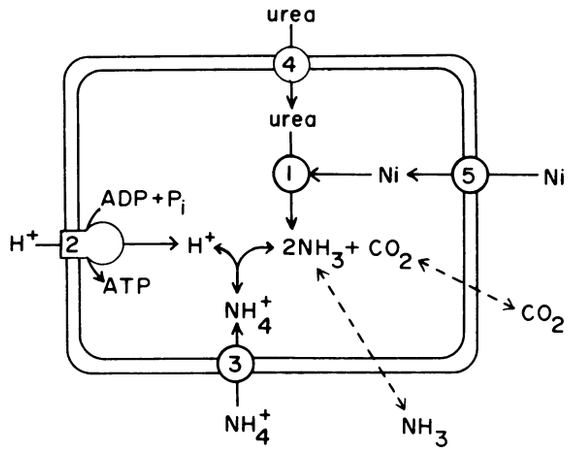

FIG. 2. Transport processes related to microbial ureolysis. Urease (protein 1) is a cytoplasmic protein; nevertheless, several membrane-bound proteins may be indirectly associated with urea degradation in certain microorganisms. For example, in *U. urealyticum* urea hydrolysis was suggested to be coupled to ATP formation (145, 190). In the proposed model, ureolysis generates a net rise in intracellular pH and the resulting proton gradient is used to drive a proton-dependent ATP synthase (protein 2). It is unclear how ammonium ion is removed from the cell in such a model. Ammonium ion transport systems (protein 3) are known to occur in numerous microbes (111), however, these transporters function in uptake of ammonium ion and are energy dependent. Ammonia and carbon dioxide can readily diffuse through the cell membrane, and no transporters are present for these molecules. In contrast, energy-dependent urea transporters (protein 4) have been found in several microorganisms (41, 88, 146, 176, 223) even though urea is able to diffuse through membranes. Finally, energy-dependent nickel transport (protein 5), often via the magnesium transporter, is necessary to generate active, nickel-containing urease (81). ADP, Adenosine diphosphate; P_i, inorganic phosphate.

assumed to be freely permeable to urea and ammonia in these assays; however, this assumption is invalid. For example, urease activity measured in cell extracts greatly exceeded the activity of intact cells for *Bacillus pasteurii* (123), *Corynebacterium renale* (126), *K. aerogenes* (103), or *Providencia rettgeri* (134). Ureolytic rates for several other microorganisms (196) appeared to be unaffected by the state of the cell; however, only very high (83 mM) levels of urea were examined. We find that ureolytic rates for intact microorganisms are generally inaccurate at low urea concentrations. The lack of free diffusion for substrate and product means that kinetic values for urease can only be obtained for permeabilized cells. In addition to sonication or other disruptive methods, permeabilization of cells can also be achieved by addition of detergents such as 0.1% hexadecyltrimethylammonium bromide (59, 126).

Energy-dependent urea transport systems (Fig. 2) have been described in yeasts (41, 176), algae (146, 223), and bacteria (88). For example, a urea transporter in *Aspergillus nidulans* was found to concentrate urea 50-fold over the medium levels, with a K_m of 30 μM urea (176). This transporter was under nitrogen regulation, being expressed at higher levels under nitrogen-deficient conditions. Thiourea was also transported by the urea transporter, and thiourea-insensitive mutants of *Aspergillus nidulans* were found to be defective in urea transport. A *Saccharomyces cerevisiae* urea transporter was also found to be nitrogen repressible and possessed a K_m of 14 μM urea (41). Similarly, K_m values of 38 and 13 μM were determined for the *Alcaligenes eutrophus* and *K. pneumoniae* urea uptake systems, and evidence was obtained that urea transport occurred in *Providencia rettgeri* and *Pseudomonas aeruginosa* (88). Interestingly, thiourea was not transported by the urea transporter in the one bacterium tested, *Alcaligenes eutrophus* (88). In contrast to all of the cases described above, no evidence was obtained for energy-dependent urea transport in studies with *Proteus vulgaris* and *Bacillus pasteurii* (88).

Energy-dependent ammonium ion transport (Fig. 2) is well characterized (111). Ammonia is able to diffuse through membranes, whereas ammonium ion is not. Internal ammonia is therefore rapidly lost from cells, and an uptake system is commonly used to maintain the required cellular levels. Thus, measurement of whole-cell ureolysis rates based on ammonia release or pH increases will be affected by the competing ammonium ion uptake system.

Ureolytic rates for whole cells in their normal environments will depend on the urea concentration, the urea transport rates, the cellular levels of urease, and the ammonium ion transport rates. In *Bacillus pasteurii* cells, which possess extremely high levels of urease but which lack a urea permease, it has been suggested that 100 mM urea is required to allow sufficient diffusion of urea through the membrane so as to support growth (88). Clearly, studies which detail the properties of purified ureases (see below) must be integrated with urea and ammonium ion transport studies to understand cellular ureolysis fully.

ENZYMOLOGY OF MICROBIAL UREASES

Various aspects of urease enzymology have been reported for many microorganisms, but few ureases have been purified or studied in detail. This section describes methods available to assay and purify ureases, and it critically examines kinetic and physical properties of purified microbial ureases. Selected properties from several ureases, summarized in Table 2, are discussed in detail below and compared with the well-characterized jack bean enzyme. In addition, the postulated mechanisms of action for several urease inhibitors will be summarized.

Methods To Assay Urease

Several excellent techniques are available to assess urease activity, including assays that monitor ammonia release, CO_2 release, or increase in pH. Several of these methods are briefly described below. Optimum assay conditions should be identified for each urease being examined; however, unless noted otherwise, a solution containing 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and 1 mM EDTA at pH 7.5 is recommended as a reasonable buffer for initial studies.

Indophenol assay. Ammonia can be reacted with phenol-hypochlorite at high pH to form indophenol, providing a simple and quantitative spectrophotometric assay (243). This very sensitive method can easily detect <0.02 μmol of ammonia, and we have found it to be very useful in detailed kinetic analyses. However, this method has the disadvantage of being a fixed-time-point assay which requires multiple samplings of each assay. Furthermore, several urease inhibitors have been found to interfere with this method.

Nesslerization reaction. The Nesslerization reaction, a fixed-time-point spectrophotometric assay, reacts ammonia with Nessler reagent, containing KI and HgI_2 (Sigma ammo-

TABLE 2. Selected properties of purified microbial ureases[a]

Microorganism	K_m (mM)[b]	Sp act (μmol of urea/min per mg of protein)[b]	Puification (fold)	pH optimum	Native M_r	Subunit M_r	Subunit composition	Metal content	Reference(s)
Bacteria									
Arthrobacter oxydans	12.5	219	121	7.6	242,000	NR[c]	NR	0.3 Ni/242,000	204
Bacillus pasteurii	40–130	1,528	66	NR	230,000	α = 65,500	α_4	0.82 –1.0Ni/α	37, 123
Brevibacterium ammoniagenes	18–72	3,570	638	7	200,000	α = 67,000	α_3	0.8 Ni/α	167
Klebsiella aerogenes	2.8	2,200	1,070	7.75	224,000	α = 72,000 β = 11,00 λ = 9,000	$\alpha_2\beta_4\gamma_4$	2.1 Ni/$\alpha\beta_2\gamma_2$	227
Mixed ruminal population	0.83	60	60	8.0	125,000	NR	NR	NR	138
Morganella morganii[d]	NR	2,431	169	NR	NR	NR	NR	NR	245
Proteus mirabilis	13	2,057	800	7.5	212,000–250,000	α = 73,000 β = 10,000 γ = 8,000	$\alpha_2\beta_4\gamma_4$	NR	21, 98
Providencia rettgeri[e]	10.5–71	30.6	43	7.5	NR	NR	NR	NR	133
Providencia stuartii[f]	9.3	5,520	331	NR	230,000	α = 73,000 β = 10,000 γ = 8,000	$\alpha_2\beta_4\gamma_4$	1.9 Ni/$\alpha\beta_2\gamma_2$	163
Selenomonas ruminantium	2.2	1,019	592	NR	360,000	α = 70,000 β = 8,000 γ = 8,000	NR	2.1 Ni/α	80, 227
Spirulina maxima	0.12	9.27	44	8.7	232,000	α = 38,000	α_6	NR	32
Staphylococcus saprophyticus	7.36	150	NR	6.8	250,000	NR	NR	NR	65
Ureaplasma urealyticum	2.5	180,000	180	7.2–7.5	380,000	α = 66,000	α_5 or α_6	NR	47, 217
	NR	65,000	NR	NR	NR	α = 76,000	NR	NR	182
	4.5	33,530	155	7.0–7.5	150,000	α = 75,000	α_2	NR	199
Fungus									
Aspergillus nidulans	1.33	670	1,031	8.5	240,000	α = 40,000	α_6	NR	42

[a] Not all of these purified preparations represent homogeneous enzymes.
[b] These values were obtained for enzymes assayed under different buffer, pH, and temperature conditions.
[c] NR, Not reported.
[d] Formerly *Proteus morganii*.
[e] Formerly *Proteus rettgeri*.
[f] Formerly *Proteus stuartii*. *P. stuartii* urease genes were expressed in *Escherichia coli*, and recombinant *P. stuartii* urease was purified from this source.

nia color reagent). Rather than the intense blue color (maximum absorbance at 625 nm) observed in the phenol-hypochlorite assay, a two- to fourfold less intense orange color develops. When high sensitivity is not required and when there is no interference by cellular components, this assay is suitable because it is rapid and easily performed.

Coupled enzyme assay. A continuous spectrophotometric assay method which uses glutamate dehydrogenase to couple ammonia release to reduced nicotinamide adenine dinucleotide oxidation has been widely used (101). This method can detect 0.003 U of urease per ml (1 U hydrolyzes 1 μmol of urea per min), which means that 0.02 μmol of ammonia would be released in 3.3 min from a 1-ml reaction. Thus, the continuous assay and the indophenol method are roughly comparable in sensitivity. However, several concerns with this coupled system have been noted (101). For example, glutamate dehydrogenase has a higher pH optimum (pH 8.3) than most ureases; the coupling enzyme may be inhibited by compounds being tested as urease inhibitors; a lag is observed in reduced nicotinamide adenine dinucleotide oxidation, requiring that the enzyme rate be approximated by a tangent to the time course; and cellular reduced nicotinamide adenine dinucleotide oxidase activity may be present in crude fractions.

An alternative coupled assay system for urease makes use of horseradish peroxidase (218). Ammonia released by urease markedly stimulates horseradish peroxidase-catalyzed peroxidation of *o*-dianisidine, resulting in an increase in absorbance at 460 nm. Two drawbacks to the method are the nonlinearity of the absorbance changes and the requirement for basic buffer conditions (pH 9.3).

pH indicator assay. Urea hydrolysis leads to a pH increase and the rate of this change can be monitored spectrophotometrically in the presence of a pH indicator such as phenol red (78, 198). This very convenient assay is suitable for routine comparison of urease rates, but is not recommended for detailed kinetic analysis. In assays containing 7 μg of phenol red per ml, a nearly linear change with time at 560 nm was observed between 0.15 and 0.5 absorbance unit when initiated at pH 6.8 (158). However, a serious drawback to this method is that the pH of the assay solution will increase during the analysis and affect enzyme activity.

Potentiometric assays. Ammonium ion production can be measured directly by using an ammonium ion-selective electrode (78, 104, 161). This method has low sensitivity (0.1 mM ammonium ion is the lower limit), and it does not provide a linear response, but it can be used in a continuous fashion. A further problem with this electrode is interference by potassium and other monovalent ions. Ammonia can also be assayed potentiometrically by using an ammonia-selective electrode. This sensitive assay can detect 10 nmol of ammonia, but it requires analysis of fixed time points because samples must be adjusted to high pH prior to measurement to fully deprotonate ammonium ion (pK_a, 9.3). Furthermore, linear response to ammonia concentration is not observed. Finally, a pH stat can be utilized to monitor directly alkalinization in a very sensitive manner (13). This excellent method is unaffected by inhibitors; however, no buffer is used in the assay and the ionic strength of the solution changes during the analysis, which may affect the activity for some ureases.

Other methods. Another method to monitor ammonia release makes use of ^{15}N-labeled urea (247). This method has been useful for selected applications, but it has little general utility and requires a mass spectrometer for analysis. Methods to measure $^{14}CO_2$ release from ^{14}C-labeled urea are widely used; however, they require access to a scintillation counter (147). The increase in conductivity as urea is hydrolyzed has also been used for assaying urease in a continuous manner (129). In addition, a little used manometric method has been described (232). Selection of the best assay method will depend on the particular experiment of interest.

Localization of urease activity in gels. One unit of urease activity can be visualized in native agarose or acrylamide gels by several methods. The most simple technique is to equilibrate the gel in a pH 6 buffer containing a pH indicator, followed by transfer of the gel to a solution containing urea (16). As the urea is hydrolyzed, the pH indicator changes color within the gel at the location of the urease activity. A photographic record of the developing gel allows visualization of bands of varied intensity, or the reaction can be quenched and the banding pattern can be permanently fixed by addition of lead acetate (208). An alternative catalytic gel stain involving precipitation of a tetrazolium salt has also been widely used (52).

Purification of Microbial Ureases

The first microbial urease purified was from *Bacillus pasteurii* in 1954 (123). Since that time, purification of several other bacterial ureases and one fungal urease has been reported (Table 2). It should be stressed that several of the ureases listed in Table 2 are not homogeneous enzymes. Each urease has been characterized to a different extent, and the degree of purification has not always been sufficiently examined.

The original purification of *Bacillus pasteurii* urease involved a series of simple fractionation steps, using ammonium sulfate, calcium phosphate, and acetone treatments (123). These compounds, a DNA precipitant such as protamine sulfate or streptomycin sulfate, and treatments with ethanol or heat have been used effectively for partial purification of many ureases. In addition, most recent urease purifications include various chromatographic separations. Interestingly, the introduction of gel filtration, anion exchange, and affinity chromatography procedures for *Bacillus pasteurii* purification (37) resulted in less active preparations than found with the original method (123). Anion-exchange resins are widely used for urease purification because every urease examined has been shown to be negatively charged at neutral pH. Hydrophobic chromatography has been useful because it binds urease more tightly than many less hydrophobic contaminants. Indeed, successive chromatography of cell extracts on diethylaminoethyl-Sepharose, phenyl-Sepharose, and fast protein liquid chromatography Mono-Q resins has been found to be a powerful combination of methods for isolating ureases from several species including *K. aerogenes* (227), *Proteus mirabilis* (21), *Providencia stuartii* (163), and *Selenomonas ruminantium* (80). Affinity chromatography by using anti-urease antibodies has been carried out successfully with *U. urealyticum* urease (182). In addition, affinity chromatography by using hydroxyurea-derivatized resin has been extremely successful for *Morganella morganii* (245), *Brevibacterium ammoniagenes* (167), and *Aspergillus nidulans* (42) ureases. A recent report describes *U. urealyticum* urease purification by using an affinity resin which "was prepared by coupling the free NH_2 group of acetohydroxamic acid with CH-Sepharose" (199). It is likely that this resin was actually prepared by using hydroxyurea as in the above three cases. Other laboratories have had little success with hydroxyurea affinity resins (37, 138; R. P. Hausinger, unpublished data), and this method may not be generally applicable.

A central step in jack bean urease purification is crystallization of the enzyme (13, 220). As an historic note, jackbean urease was the first enzyme successfully crystallized. In contrast, crystallization has been less useful for purifying microbial ureases. Urease crystals have been reported for the *Providencia rettgeri* enzyme; however, only low levels of activity were retained (133).

Urease Stability

Ureases from several sources have been found to be inactivated by heavy metals (15, 65, 133, 138, 167) and by oxidation (126, 133, 137); thus, most purification procedures include low levels (e.g., 1 mM) of ethylenediaminetetraacetate (EDTA) and a thiol such as dithiothreitol or 2-mercaptoethanol to preserve activity.

When stored at 0°C in 50 mM HEPES, pH 7.5, buffer containing 1 mM EDTA and 1 mM 2-mercaptoethanol, purified urease from *K. aerogenes*, *Proteus mirabilis*, *Providencia stuartii*, and *Sporosarcina ureae* have been found to retain nearly 100% activity for >1 month (Hausinger, unpublished observation). Glycerol further stabilizes urease activity, as shown in studies with *Brevibacterium ammoniagenes* urease (167). Approximately 70% of urease activity was retained after 2 months at 20°C in 50% glycerol–1 mM EDTA, 5 mM 2-mercaptoethanol–50 mM phosphate buffer at pH 7.5. In the absence of glycerol, 90% of the activity was lost (167). *U. urealyticum* urease was fully stable for >20 days when stored at 4°C in pH 7.2 buffer containing 1% bovine serum albumin (217); it is not clear whether the added albumin was required.

The pH and temperature stabilities for several ureases have been studied. Activity was irreversibly lost when ureases from *Arthrobacter oxydans* and *Bacillus pasteurii* were exposed to pH values below 5 and 5.2, respectively (123, 204). In the former case, loss of activity was correlated with nickel removal from the enzyme (see subsection, "Nickel Active Site Studies"). *Proteus mirabilis* urease activity was stable for 24 h at pH values of 7 to 10 at 0°C, whereas at 37°C 24-h stability was only observed between pH 8 and 9 (21). Full activity was retained after 5 h at 30°C for *Brevibacterium ammoniagenes* for pH values between 5 and 10, with sharp decreases in activity at each pH extreme (167). At pH 7.5, the *Proteus mirabilis* and *Selenomonas ruminantium* enzymes were stable for 10 min to a temperature of 60°C, but were rapidly inactivated at higher temperatures (Hausinger, unpublished data). Similarly, purified urease from *Brevibacterium ammoniagenes* (167) and a crude enzyme preparation from *Alcaligenes eutrophus* (88) were stable for 10 min in pH 7.5 and 7.0 buffers at temperatures exceeding 50°C. Interestingly, *Providencia rettgeri* urease was stable to 80°C in the presence of urea, but activity was rapidly lost when substrate was absent (133).

Purified *K. aerogenes* urease in 50 mM HEPES (pH 7.75) at 0°C was found to be stable in the presence of a wide range of compounds. Little loss of activity was observed when the enzyme was stored for 200 h with 5% Triton X-100, 1% sodium dodecyl sulfate, 20% ethylene glycol, 20% glycerol, 200 mM 2-mercaptoethanol, or 1 M KCl (227). Furthermore, this enzyme, as well as those from *Proteus mirabilis* (21) and *Selenomonas ruminantium* (80), tolerated 10% dimethyl sulfoxide in the buffer. For a variety of partially purified ureases, most activity was retained after precipitation steps involving acetone (102, 123, 167, 204). In contrast, ethanol crystallization led to low recovery of *Providencia rettgeri* urease activity (133).

In summary, microbial ureases are quite stable in the presence of EDTA and thiol protectants when they are not subjected to pH extremes or high temperature. This feature facilitates their characterization, as discussed below.

Kinetic Properties of Microbial Ureases

K_m values of ureases. This review focuses only on urea as a substrate. Nevertheless, it is known that acetamide, formamide, *N*-methylurea, semicarbazide, and hydroxyurea all serve (albeit poorly) as substrates for jack bean urease (1, 14) and may also be substrates for microbial ureases. We have found that the urea K_m values of purified ureases agree quite well with the values observed for crude cell extracts. Thus, K_m values can be obtained from crude urease preparations. However, it must be kept in mind that some buffers (discussed in the section on urease inhibitors) inhibit urease and lead to inaccurate K_m determinations.

Individual ureases possess K_m values ranging from 0.1 to >100 mM urea (Table 2). For comparison, jack bean urease possesses a K_m of 2.9 mM (14). A range of K_m values is reported for *Bacillus pasteurii* (123), *Brevibacterium ammoniagenes* (167), and *Providencia rettgeri* (133) because the K_m was reported to vary with buffer and pH conditions. In contrast, ureases from *K. aerogenes* (227) and *Proteus mirabilis* (21) exhibited nearly pH-independent K_m values from pH 5 to 11 when noninhibitory buffers were used. Different strains of the same species may possess very different K_m values; for example, various strains of *Proteus mirabilis* exhibited K_m values ranging from 13 to 60 mM (21, 97).

Ureolytic microorganisms which infect the urinary tract, such as *Proteus mirabilis*, *Morganella morganii*, and *U. urealyticum*, have ureases which are clearly saturated in this 0.4 to 0.5 M urea environment (76). Urea concentration in a defined-microbe rumen is approximately 5 mM (34); thus, in a rumen containing ureolytic microbes such as *Selenomonas ruminantium* the ureases are unlikely to be fully saturated. Similarly, *C. pylori* urease has a K_m of 0.8 mM, which means that urea diffusing from the bloodstream (1.7 to 3.4 mM urea) is unlikely to completely saturate this enzyme. Environmental urea concentrations can vary widely, and it is unclear whether any correlation exists between typical urea levels and K_m values of soil microbes such as *Arthrobacter oxydans*, *Bacillus pasteurii*, and *Brevibacterium ammoniagenes* or aquatic microbes such as *Spirulina maxima*. Any relationship between urease K_m values and environmental urea concentrations is further complicated by the presence or absence of a urea permease in various microorganisms (see subsection, "Urea and Ammonia Ion Transport").

Urease specific activities. The specific activity determined for any enzyme depends on the state of purification, on the specific activity of the homogeneous protein, and on the conditions of assay. For example, homogeneous jack bean urease assayed under standard conditions of pH 7.0 and 38°C has a specific activity of approximately 3,500 μmol/min per mg (1). The specific activities for several purified microbial ureases, when assayed by using different standardized conditions, are the same magnitude as that of the plant enzyme, ranging from 1,000 to 5,500 μmol/min per mg. These include ureases isolated from *Bacillus pasteurii* (37, 123), *Brevibacterium ammoniagenes* (167), *K. aerogenes* (227), *Morganella morganii* (245), *Proteus mirabilis* (21), *Providencia stuartii* (163), and *Selenomonas ruminantium* (80) (Table 2).

In contrast to the microbial ureases with specific activities comparable to that of the jack bean enzyme, ureases purified

from *Arthrobacter oxydans* (204), *Providencia rettgeri* (133), *Spirulina maxima* (32), *Staphylococcus saprophyticus* (65), *Aspergillus nidulans* (42), and from a mixed ruminal population (138) exhibited significantly lower specific activities. These ureases may simply possess intrinsically lower activity, the enzymes may have been partially inactivated during purification, or the purifications may have been incomplete. The latter explanation is probably correct for *Spirulina maxima* urease which was purified only 44-fold by using a simple three-step procedure (32). Accordingly, it may be worthwhile to reexamine this and other ureases of low specific activities.

The specific activity of *U. urealyticum* urease deserves special mention. Three recent publications have reported specific activities for highly purified preparations ranging from 33,530 to 180,000 μmol/min per mg of protein (182, 199, 217). In addition, the specific activity of crude cell extracts was shown to be higher than that of crystalline jack bean urease by other investigators (15). These results suggest that the *U. urealyticum* enzyme is significantly different from other microbial ureases.

The specific activity of urease is strongly pH dependent, and the optimum pH for each purified enzyme is shown in Table 2. In several cases (65, 102, 167), pH effects must be viewed with caution because of inhibition by thiols, borate, phosphate, and other buffer components (see subsection, "Urease Inhibitors"). In other cases, pH dependence studies were carried out with buffers that exhibited insignificant urease inhibition. For example, kinetic analysis of *Proteus mirabilis* and *K. aerogenes* enzymes (21, 227) revealed that pH affects the V_{max} and demonstrated the importance of two titratable groups in the enzyme. One amino acid residue must be deprotonated for maximum activity and possessed a pK_a value of 6.25 for *Proteus mirabilis* urease and 6.55 for *K. aerogenes* urease. A second group must be protonated for maximum enzyme activity and exhibited pK_a values of 9.2 to 9.4 and 8.85 for the *Proteus mirabilis* and *K. aerogenes* ureases, respectively (21, 227). The identity of, and catalytic role for, these two titratable groups in urease have not been elucidated.

Urease Structural Properties

Subunit molecular weights. Subunit characterization of bacterial ureases has led to conflicting results. Ureases from *K. aerogenes* (227), *Proteus mirabilis* (21, 227), *Providencia stuartii* (163), and *Selenomonas ruminantium* (80, 227) have been found to possess three distinct types of subunits with M_rs of 68,000 to 73,000 (α), 8,000 to 12,000 (β), and 8,000 to 10,000 (γ). In contrast, a single subunit type was reported for ureases isolated from *Bacillus pasteurii* (37), *Brevibacterium ammoniagenes* (167), *Spirulina maxima* (32), and *U. urealyticum* (182, 199, 217).

One explanation for the discrepancy in the number of bacterial urease subunits is that small subunits may have been inadvertently overlooked in the putatively "homopolymeric" enzymes. For example, denatured *Selenomonas ruminantium* urease was initially reported to contain a single, M_r-72,000 subunit when analyzed by using a 7.5% polyacrylamide gel (80). However, when the same preparation was examined by using a 10 to 15% gradient gel, three polypeptides were observed (227). The small peptides are poorly resolved from the dye front on gels containing ≤10% acrylamide and are easily missed. It is also possible that true differences exist in the number of urease subunits among the different bacteria. For example, the apparently homopolymeric ureases were isolated from gram-positive microorganisms, whereas gram-negative microbes all appear to possess ureases with three distinct subunit types. Nevertheless, preliminary studies of urease from *Sporosarcina ureae*, a gram-positive bacterium, suggested a multicomponent enzyme (227). Given the exceptionally high activity of *U. urealyticum* urease, this enzyme may belong to a distinct urease subclass which could be homopolymeric. However, the subunit size for the mycoplasmal enzyme is unclear, with reports of M_rs of 76,000 (182), 75,000 (199), and 66,000 (217).

Ureases have been reported to possess a single subunit in fungi, including the enzymes from *Ustilago violacea* (subunit M_r, 80,000; no details were provided and the enzyme was not purified) (4) and *Aspergillus nidulans* (subunit M_r, 40,000) (42). These results are consistent with that of the best-studied eucaryotic urease from jack bean which has been shown to possess a single type of subunit (1, 14) with a molecular weight of 90,770, as shown by protein sequencing (139, 224).

Native molecular weights. Molecular weights for native urease are typically measured by gel filtration chromatography. Results from such studies can be affected by enzyme aggregation or interaction with the resin. In general, purified microbial ureases exhibit a native M_r of 200,000 to 250,000 (Table 2). An anomalously low value (M_r, 125,000) was reported for urease isolated from a mixed ruminal population (138), whereas purified urease from *Selenomonas ruminantium* (80) was found to possess a high molecular weight (M_r, 360,000). High native molecular weight values of up to 800,000 M_r were reported for crude preparations of enzyme from several strains of *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *Providencia rettgeri*, and *Providencia stuartii* (97, 196); however, many of these values are due to enzyme aggregation with contaminants. This aggregation is sensitive to ionic strength and can be minimized by including 0.1 M NaCl in the buffers (98, 157).

Urease molecular weights have also been determined by using native gel electrophoretic methods. Results of this independent measurement agreed with the value of approximately 225,000 M_r for ureases from *K. aerogenes* (227) and *Proteus mirabilis* (98). Partially purified *U. urealyticum* ureases were found to be quite large (M_r, 380,000) by this method, in agreement with one value (M_r, 330,000) (47), but in contrast to a second value (M_r, 150,000) (199), obtained by using gel filtration chromatography.

All purified microbial ureases were found to possess molecular weights which were much smaller than that of the jack bean enzyme (M_r, 590,000) (1). This feature is important because it may simplify eventual X-ray crystallographic analysis or other techniques which depend on the molecular dimensions.

Subunit stoichiometry. The homopolymeric jack bean urease has clearly been shown to possess an α_6 structure (i.e., six copies of identical subunits) based on the molecular weights of the denatured subunit and the native enzyme (1). A similar hexameric structure was reported for urease from *Aspergillus nidulans* (42), although the size of the fungal subunit was less than half that of the plant urease.

The native bacterial enzyme structure has been reported to be trimeric for *Brevibacterium ammoniagenes* (167), tetrameric for *Bacillus pasteurii* (37), hexameric for *Spirulina maxima* (32), and dimeric (199) to hexameric (217) for *U. urealyticum*. The initial report for *Selenomonas ruminantium* urease indicated a pentameric structure (80); however, this enzyme was recently shown to possess three subunit types (227) and the stoichiometry is not established. Simi-

larly, the other homopolymeric bacterial ureases may need to be reexamined as discussed above.

In contrast to the above results, ureases from *K. aerogenes* (227), *Proteus mirabilis* (J. M. Breitenbach and R. P. Hausinger, unpublished data), and *Providencia stuartii* (163) are clearly composed of three subunit types in a probable $(\alpha_1\beta_2\gamma_2)_2$ stoichiometry. The subunit compositions are somewhat uncertain because they are based on Coomassie blue staining intensity of bands in polyacrylamide gels. A better method to confirm the subunit ratios would be to isolate the protein from cells grown on ^{14}C-labeled substrate and quantitate the radioactivity incorporated into each band. The individual subunits have not been isolated without the use of denaturants, and the role for each subunit is unknown.

Isoelectric point. By using isoelectric focusing techniques, the pI has been measured for several purified microbial ureases. A single form was observed for *Bacillus pasteurii* and *Brevibacterium ammoniagenes* ureases at pI values of 4.6 and 4.1, respectively (37, 167). In contrast, four bands were observed (pI, 4.3 to 4.7) for *Arthrobacter oxydans* urease (204) and two or three bands (pI, 5.0 to 5.2) for the cell extracts from *U. urealyticum* (47, 199). In addition, analysis of cell extracts from *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *Providencia rettgeri*, and *Providencia stuartii* (97) revealed that each species possessed a major form of urease (pI, 5.1 to 5.9) and at least one secondary form. Isoelectric focusing results must be interpreted with caution because microbial ureases are irreversibly inactivated at low pH values and artifactual pI values may have been generated. Nevertheless, an acidic pI of 4 to 6 was consistently observed in all determinations.

"Isoenzyme" multiplicity. Cell extracts from *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *Providencia rettgeri*, and *Providencia stuartii* (97, 207), among many other microorganisms, have been reported to yield multiple bands of urease activity when analyzed by using native polyacrylamide gel electrophoresis. In most cases, electrophoresis results in a major band of activity and one or two less intense and more slowly migrating bands. Urease purification generally leads to an enrichment of the major band of activity; however, several reports have described secondary peaks of activity which may represent alternate forms of the enzyme (21, 163). The different bands of urease activity are not due to expression of multiple isozymes from different urease genes. For example, mutations in the urease genes for *Providencia stuartii* (163) or *Proteus mirabilis* (98) eliminated all urease activity, rather than eliminating a single band from the gels. In addition, recombinant ureases which were cloned from *K. aerogenes* (S. B. Mulrooney and R. P. Hausinger, unpublished data), *Proteus mirabilis* (98, 240), and *Providencia stuartii* (158) still exhibited multiple urease bands. Interestingly, recombinant *Proteus mirabilis* urease yielded an inverted intensity pattern on native gels, with the slowest migrating band being the most intense (240).

A multiplicity of active bands has also been reported for samples of crystalline jack bean urease (16) in which the multiple forms have been shown to represent different urease aggregation states, including the major hexameric structure, a trimeric form, and a series of higher-molecular-weight aggregates (1). Many of these states were interconvertible, depending on the pH, the presence of salt, glycols, or thiols, or other changes in the buffer. In contrast to these results, the multiplicity of bands observed for microbial ureases cannot be explained by simple self-aggregation. By using several gels of varied polyacrylamide percentages, the molecular weights for the two major bands of *K. aerogenes* and

FIG. 3. Partial amino acid sequences of bacterial and plant ureases. (A) Amino-terminal 19 residues of the large subunit (M_r, 72,000) of *K. aerogenes* urease (Todd and Hausinger, unpublished data). (B) First 19 amino acids of the *Proteus mirabilis* urease large subunit (M_r, 73,000) deduced from the partial nucleotide sequence of the gene (Jones and Mobley, unpublished data). (C) Internal sequence of jack bean urease (M_r, 90,770) from residues 271 to 290 (224). Sequence identities are enclosed.

Proteus mirabilis were estimated. No significant differences in molecular weight were observed for the different urease forms (J. M. Breitenbach, M. J. Todd, and R. P. Hausinger, unpublished). It is possible that small differences in native molecular weight did exist, such as a gain or loss of one of the small subunits. However, no consistent changes in subunit stoichiometry were observed for the different forms of *K. aerogenes* urease isolated from native gels and rerun on sodium dodecyl sulfate gels (M. J. Todd and R. P. Hausinger, unpublished data). Attempts to alter the banding pattern by incubating the enzyme at varied pH values or in the presence of thiols, detergents, salts, glycols, guanidine-HCl, urea, ammonia, nickel ion, solvents, alkaline phosphatase, or neuraminidase have not been successful (Breitenbach and Hausinger, unpublished data). The basis of the multiple forms of microbial urease observed in native gels remains unknown.

Amino acid composition. The amino acid composition has been reported only for microbial ureases isolated from *Brevibacterium ammoniagenes* (167) and *K. aerogenes* (227). The analyses were compared with each other and each was compared with that of jack bean urease. The microbial ureases were found to be more closely related to each other than either was to the plant enzyme (227). No remarkable features were noted in the compositions.

Sequence analysis. The amino-terminal sequence of the large subunit (M_r, 72,000) from the *K. aerogenes* enzyme is shown in Fig. 3A. Since the sequence does not begin with methionine, proteolytic processing must have occurred. The amino terminus of the corresponding *Proteus mirabilis* subunit was deduced from the partial nucleotide sequence of the gene (Fig. 3B). The amino-terminal methionine immediately precedes a stretch of 18 residues in which 15 match the *K. aerogenes* sequence. There is identity for 10 of 19 residues between each bacterial sequence and an internal sequence from jack bean urease (139, 224; Fig. 3C). This sequence homology will be further discussed in terms of gene organization in subsection, "Bacterial Urease Operon." The only other information on microbial urease amino acid sequence is for *Brevibacterium ammoniagenes*, in which the amino terminus was identified by dansylation to be a methionine and the carboxyl terminus was shown to be a leucine by using carboxypeptidase A treatment (167).

Crystal structure. Crystallization of *Providencia rettgeri* urease has been reported (133); however, the crystals had low urease activity and were unsuitable for further analysis. Despite the fact that jack bean urease was the first enzyme to be crystallized, the three-dimensional structure of the plant urease has not been determined.

Nickel Active Site Studies

Nickel is generally not considered an important biological metal; however, this ion was shown in 1975 to be an essential

component of jack bean urease (1, 46). The first indications of an association between nickel ion and microbial urease were reports of a nickel dietary requirement for lamb ruminal urease activity (214, 215). Several microorganisms have since demonstrated a nickel requirement for urease activity or have been shown to possess nickel-containing ureases (81). Recent studies, summarized below, have focused on characterizing the enzyme metallocenter.

Nickel content of microbial ureases. Although there is compelling evidence for the presence of nickel in microbial urease (81), few purified enzymes have been subjected to nickel quantitation. Ureases from *K. aerogenes* (227) and *Providencia stuartii* (163) possess approximately two nickel ions per $\alpha\beta_2\gamma_2$ structure, or four nickel ions per native enzyme molecule. In contrast, a single nickel ion per polypeptide was found in *Brevibacterium ammoniagenes* and *Bacillus pasteurii* ureases, which are reported to possess a homomeric structure (37, 167). It is possible that insufficient nickel ion was present in the growth medium for the latter two cultures and that approximately equal amounts of apourease copurified with urease. No nickel ion was added to the *Brevibacterium ammoniagenes* medium (167), and only 80 nM nickel ion was added to the *Bacillus pasteurii* medium (37).

In jack bean urease, two nickel ions were shown to be present per catalytic unit by quantitating the nickel ion present and the amount of ^{32}P-radiolabeled phosphoramidate inhibitor which bound to the enzyme (46). Inhibition analysis of *K. aerogenes* urease by using the tight-binding inhibitor phenylphosphorodiamidate demonstrated the presence of one active site per $\alpha\beta_2\gamma_2$ unit (Todd and Hausinger, unpublished data). Thus, the multimeric bacterial ureases possess two nickel ions per active site as in the plant enzyme.

Urease nickel ion is tightly bound to the protein as evidenced by retention of the metal ion during enzyme isolation in buffers containing 1 mM EDTA. However, nickel can be released from urease under acidic conditions, leading to irreversible loss of activity, as shown in studies with the *Arthrobacter oxydans* enzyme (204). Inactive, nickel-free urease protein was also produced when the algae *Phaeodactylum tricornutum* and *Tetraselmis subcordiformis* (186), the cyanobacterium *Anabaena cylindrica* (130), the purple sulfur bacteria *Thiocapsa roseopersicina* and *Chromatium vinosum* (6), or the enteric bacterium *K. aerogenes* (Mulrooney and Hausinger, unpublished data) was grown in nickel-depleted medium. Urease activity was generated in these cells in the absence of protein synthesis (i.e., by treatment with chloramphenicol or cyclohexamide) when nickel ion was added. It was not established whether apoenzyme or inactive, metal-substituted urease was produced under the nickel-free conditions, and the inactive proteins have not been purified. No successful in vitro reconstitution of nickel-free protein has been described, and the mechanism of nickel ion insertion into urease protein is unknown. Interestingly, nickel ion was found to partially activate urease activity in crude extracts of the lichen *Evernia prunastri* (179). It is not clear whether the lichen synthesized nickel-deficient enzyme or an allosteric activation occurred.

Characterization of the urease nickel center. The nickel center in microbial urease gives rise to a weak ultraviolet visible absorbance spectrum superimposed on the spectrum of the protein. At protein concentrations of approximately 40 mg/ml, this spectrum was shown to possess an absorbance peak at 406 nm for the *K. aerogenes* enzyme (Todd and Hausinger, unpublished data). Upon addition of 2-mercaptoethanol, the absorbance intensity increased at 322, 374, 432, and 750 nm, presumably due to charge transfer complex formation between the thiol sulfur and the nickel ion. The finding that a thiol binds to nickel ion is significant, because thiols are competitive inhibitors of urease (see next subsection), which means that urea must then also bind to nickel ion. The spectral features are nearly identical to those reported for the jack bean urease (1, 14).

Several important aspects of the microbial urease metallocenter remain unclear. For example, it is not established whether the two nickel ions per active site interact as a single bimetal center or as two independent sites. The Ni-Ni distance has not been determined. The ligands to the nickel ions are unknown. Present efforts are aimed at characterizing the urease active site by using biophysical methods, including magnetic susceptibility measurements, low-temperature magnetic circular dichroism spectroscopy, and X-ray absorption spectroscopy.

Urease Inhibitors

Microbial urease inhibitors have potential value in controlling urolithiasis (194) and in enhancing the efficiency of urea fertilizers (164). Furthermore, urease inhibitors can be used as probes to unravel the enzyme mechanism. Although few detailed kinetic or mechanistic studies have been reported, a summary of the general classes of urease inhibitors is provided below. In Table 3, the mechanisms of inhibition and the inhibition constants for selected compounds are given for a single purified urease, that of *K. aerogenes*.

Substrate analogs. Several urea analogs have been examined as urease inhibitors, including alkylated ureas, various thioureas, hydroxyurea, and numerous hydroxamic acids. Most substrate analog studies have been carried out with whole cells or crude enzyme preparations rather than with purified enzymes. Substituted ureas and thioureas are discussed here, and hydroxyurea and hydroxamic acids are discussed separately below.

The effects of alkylated ureas on urease activity have been examined in cell extracts from *Corynebacterium renale* (126), *K. aerogenes* (103), *Proteus vulgaris* (180), and several other enteric bacteria (196). No significant urease inhibition was observed for any of the cell extracts by urea analogs such as methylurea, ethylurea, and phenylurea. Phenylurea was shown to be a poor competitive inhibitor with a K_i of 94 mM for purified *Brevibacterium ammoniagenes* urease (167). Mixed inhibition by methylurea was observed in the case of *K. aerogenes* urease (Table 3).

Despite the close structural similarity between urea and thiourea, the latter compound is not a substrate for urease. By studying either whole cells or cell extracts, urease activity was found to be inhibited by 100 mM thiourea in *Corynebacterium renale* (126) and 0.2 mM in *Proteus vulgaris* (180), whereas substituted thioureas were generally much poorer inhibitors of urease (180). Although 0.65 mM thiourea was reported to inhibit cell extracts of *K. aerogenes* (103), no significant inhibition was observed for a purified preparation of *K. aerogenes* urease at levels exceeding 25 mM thiourea (Table 3). A similar lack of inhibition was noted with isolated *Brevibacterium ammoniagenes* urease (167) and with crude preparations of urease from a mixed ruminal population (137).

Hydroxyurea. Hydroxyurea is both an inhibitor and a substrate of microbial ureases (138, 196). Addition of this compound to a sample of urease results in rapid inhibition, followed by a slow recovery of activity as hydroxyurea is hydrolyzed. Inhibition of purified *Brevibacterium ammoni-*

TABLE 3. Inhibitors of purified *K. aerogenes* urease[a]

Class of compound	Inhibitor	Mechanism	K_i (mM)
Substrate analog	Methylurea	Mixed	≫25
	Thiourea	ND[b]	≫25
Hydroxamic acid	Acetohydroxamic acid	Competitive[c]	2 μM
Phosphoroamide	Phenylphosphorodiamidate	Competitive[d]	<0.1 nM
Phosphate	H_3PO_4[e]	Competitive	0.12 μM
Thiols	2-Mercaptoethanol	Competitive	0.55
Boron containing	Boric acid	Competitive	0.33
Halogen	Fluoride	Complex[f]	0.48
Inactivators[g]			k_{+1} (M^{-1} s^{-1})
Alkylating agent	Iodoacetamide		0.0276
	Iodoacetic acid		0.0011
	N-Ethylmaleimide		5.04
Disulfide	DTNB[h]		0.287

[a] All studies were carried out in pH 7.75 buffer at 37°C by Todd and Hausinger (unpublished data). Methylurea inhibition studies were performed by Breitenbach and Hausinger (unpublished data).
[b] ND, Not determined.
[c] Slow-binding inhibitor. Non-steady-state kinetic methods must be used to characterize this inhibition fully.
[d] A slow, tight-binding inhibitor requiring the use of non-steady-state kinetic methods. In addition, this compound was suggested to be a substrate of jack bean urease (2).
[e] The deprotonated compound inhibits poorly; a total phosphate concentration of approximately 250 mM is required to provide 0.12 μM H_3PO_4 at this pH.
[f] This compound slowly binds to a state of the enzyme which occurs only during catalysis.
[g] For inactivators, K_i or I_{50} values are meaningless. Rather, the pseudo-first-order rate constants of the reactions are provided.
[h] DTNB, 5,5′-Dithiobis(2-nitrobenzoic acid).

agenes urease was reported to be competitive, with a K_i of 0.23 mM (167). A K_i of 0.125 mM was obtained for *Aspergillus nidulans* urease (42), but the mechanism of inhibition was not reported. No detailed kinetic analysis of the interaction between this compound and microbial urease has been published.

Hydroxamic acids. Hydroxamic acids were shown in 1962 to be potent inhibitors of jack bean urease (112). Since that time, a wide range of hydroxamic acids has been examined as inhibitors of microbial ureases (62, 79, 113, 165, 169). The best-studied hydroxamate is acetohydroxamic acid, which has been shown to inhibit ureases from *Clostridium sordelli*, *E. coli*, *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *Providencia rettgeri*, *Staphylococcus aureus* (196), and many other microorganisms, as well as ureases from soil (183). Hydroxamic acids are good metal chelators; thus, the mechanism of inhibition has been generally assumed to involve binding to the active site nickel ion. Spectroscopic evidence obtained for jack bean urease is consistent with this assumption (1).

Most inhibition studies with hydroxamic acid have been improperly carried out from an enzyme kinetics standpoint. For example, some investigators have used Lineweaver-Burk plots to deduce the K_i and reported noncompetitive inhibition for hydroxamic acids preincubated with enzyme (54, 62, 196). This approach is inappropriate for kinetic analysis because of the time-dependent nature of hydroxamic acid binding and the very slow inhibitor dissociation rate. Alternatively, many laboratories have reported hydroxamic acid I_{50} values, the amount of inhibitor needed to inhibit the enzyme by 50% (62, 79, 113, 157, 165, 196). These studies typically yield I_{50} values in the micromolar range, and they can be compared within a single class of inhibitors if all experiments are performed under the same reaction conditions and equilibrium is reached in all cases. The I_{50} value is related to K_i; however, the relationship between these values will depend on the type of inhibition, the substrate concentration, and the enzyme concentration (35, 229). In many reports it is not clear that equilibrium conditions were obtained, and experiments have often lacked sufficient detail for complete kinetic characterization. In contrast, proper kinetic analysis of hydroxamic acid inhibition has been carried out with jack bean urease (1). By measuring rates of urease inactivation under varied conditions, and by measuring reactivation rates after removal of free inhibitor, acetohydroxamic acid was found to be a reversible, competitive inhibitor of this enzyme, with a slow dissociation rate (k_{-1}, 8.4×10^{-5}/s) and a K_i of approximately 4 μM at pH 7.0 to 7.1 and 25°C (1). It is worth noting that, at inhibitor concentrations equal to the K_i, several hours are required for equilibrium to be reached. Analogous studies have now been carried out with *K. aerogenes* urease (Table 3), with similar results.

Phosphoroamides. A variety of phosphoroamide compounds are extremely potent inhibitors of urease (114, 125, 143, 155). For example, several studies have demonstrated the inhibition of urease activity in soils by phenylphosphorodiamidate (30, 143, 185, 228). Amido derivatives of phosphoric and thiophosphoric acids (125) and *N*-acyl derivatives of phosphoric triamides (143) are also effective inhibitors of soil urease. Several *N*-acyl phosphoric triamides were shown to greatly inhibit ureases from whole cells or cell extracts of *Morganella morganii*, *Mycobacterium smegmatis*, *Proteus mirabilis*, *Proteus vulgaris*, *Providencia rettgeri*, and *U. urealyticum* (106, 114, 155, 225).

Phosphoroamide compounds possess a tetrahedral geometry that may mimic an intermediate state in enzymatic catalysis, thus acting as transition state analogs. For jack bean urease there is spectroscopic evidence that phosphoramidate binds to the active site nickel (1). The inhibitory potency of phosphoroamides are generally expressed in terms of their I_{50} values. As described for the hydroxamates, I_{50} values are not a good measure of efficacy, because they vary with equilibration period, substrate concentration, and enzyme concentration. The reported I_{50} values are in the nanomolar range, which means that the inhibitor concentration may approach the enzyme concentration. Proper kinetic studies are difficult to carry out, because these compounds are apparently slow, tight-binding inhibitors and the detailed kinetics have not been fully elucidated. To further compli-

cate kinetic analysis, phenylphosphorodiamidate and several phosphorotriamide derivatives were suggested to be hydrolyzed by the plant enzyme to yield diamidophosphate, which may be the actual inhibitor (2).

Phosphate. Recent studies with *K. aerogenes* urease have indicated that fully protonated phosphoric acid is a competitive inhibitor with a K_i of 0.12 μM (Table 3). Little inhibition is noted at neutral pH because partially deprotonated phosphate binds poorly to the enzyme. For example, a total concentration of approximately 250 mM phosphate is required to yield 0.2 μM H_3PO_4 at pH 7.75. Phosphate inhibition may account for some of the anomalous pH-dependent behavior reported for urease activity in other microorganisms (65, 102, 123, 167). Thus, phosphate buffers must be used cautiously, especially at low pH, for kinetic analysis of urease.

Thiols. Thiols have been shown to competitively inhibit ureases from jack bean (1) and *K. aerogenes* (Table 3). In both cases, spectroscopic evidence demonstrated that 2-mercaptoethanol formed a charge-transfer complex with the active site nickel ion. Since nickel-sulfur interaction is observed with a competitive inhibitor, this provides strong evidence that urea binds to nickel ion. Although thiols aid in enzyme stabilization, they should not be included in urease assay buffer.

Boric acid and boronic acids. Boric acid and boronic acids are competitive inhibitors of *Proteus mirabilis* urease, with K_i values in the range of 0.1 to 1.2 mM at pH 7.5 (21). By characterizing the pH dependence of the inhibition constant for boric acid, it was suggested that the uncharged trigonal borates are the inhibitory species. As in the case of serine proteases, some active site group may covalently bind to these compounds in a reversible manner to form a tetrahedral complex (21). Alternatively, boric acid and boronic acids may bind to the metal center.

The inhibition by boric acid may explain some anomalous results in studies in which boric acid buffers were used (102). Care should be taken to correct for borate inhibition when this compound is used as a buffer.

Fluoride. Fluoride was known to be an inhibitor of ruminal urease since 1943 (177). The kinetic mechanism of this inhibition is still not fully characterized; however, fluoride apparently binds slowly and reversibly to a form of the enzyme which occurs only during catalysis. The K_i value determined for fluoride was 0.4 mM (Table 3), whereas chloride salts show little inhibition at 1 M concentrations.

Thiol reactive reagents. Reagents which specifically react with thiol groups are known to inactivate many enzymes, including urease. For example, the alkylating agents *N*-ethylmaleimide, iodoacetamide, and iodoacetic acid have been shown to inhibit several microbial ureases (138, 167, 199; Table 3). Similarly, the thiol-specific reagent *p*-chloromercuribenzoate has been found to inactivate ureases from *Brevibacterium ammoniagenes* (167), a mixed ruminal population (138), and *U. urealyticum* (199). These compounds are irreversible inhibitors; thus, values of K_i and I_{50} have no meaning. Furthermore, contaminating compounds such as other proteins or buffer components can react with these reagents and decrease the inhibitory effects toward urease. One method to assess the effectiveness of these compounds as urease inhibitors is to measure the apparent first-order loss of activity at various reagent concentrations and calculate the pseudo-first-order rate constants of inactivation. Such values have been obtained in studies with purified *K. aerogenes* urease (Table 3).

Disulfide reagents such as 5,5′-dithiobis(2-nitrobenzoic acid) (Elman's reagent) also inactivate ureases by a first-order process (167) (Table 3). In light of the results obtained with thiol-specific reagents and from disulfide inactivation studies, a reasonable conclusion is that microbial ureases possess an essential thiol. This is consistent with the reports for the jack bean enzyme (1).

MOLECULAR BIOLOGY OF UREOLYSIS

Molecular biological studies have recently begun to elucidate the organization and function of the complex urease operon in bacteria. These studies include the demonstration of plasmid-encoded urease genes in some bacteria, the cloning and expression of bacterial urease genes, and the partial definition of several bacterial urease operons. In contrast to the bacterial urease studies, molecular biological characterization of eucaryotic microbial ureases has been more limited. Each of these topics will be discussed below. In addition, plant urease molecular biology will be summarized for comparison.

Plasmid-Encoded Ureases

Most bacterial ureases appear to be chromosomally encoded; however, several examples of plasmid-borne urease genes have been reported and are described below.

Providencia stuartii. The urease of *P. stuartii* first gained notoriety because of a puzzling taxonomic problem. In an epidemiologic study by Penner et al. (178), isolates from bacteriuric patients were observed to vary in expression of urease. Although all isolates shared the same *Providencia* O serotype and 17 other biochemical markers, the shift to a urease-negative phenotype required that an organism identified as *Proteus rettgeri* now be called *Providencia stuartii*. It was postulated that urease genes resided on plasmid DNA and had been spontaneously cured. Shortly thereafter, Farmer et al. (49) made a similar observation in that a blood culture from a bacteremic patient yielded urease-positive *P. rettgeri* and urease-negative *P. stuartii*, both with the same unusual antibiotic resistance pattern. Furthermore, *P. stuartii* isolates were "spontaneously generated" from single colonies of the *P. rettgeri* strain. Supporting their case with DNA-DNA hybridization data, Farmer et al. recommended that urease-positive *P. rettgeri* biogroup 5 be reclassified as urease-positive *P. stuartii* and also hypothesized that the enzyme was plasmid encoded.

Transfer of urease genes from *P. stuartii* to *E. coli* by a 140-megadalton conjugative plasmid was unambiguously demonstrated by Grant et al. (69) and later corroborated by Hollick et al. (86) and Mobley et al. (156). As a consequence of these reports, it was generally accepted that the ureases of *P. stuartii* were always plasmid encoded. However, a urease gene probe that hybridized strongly with whole-cell DNA from *P. stuartii* isolates hybridized with plasmid DNA from only 1 of 20 strains. The urease genes were chromosomally encoded in the remaining isolates (H. L. T. Mobley, unpublished observation).

E. coli. In 1979, Wachsmuth et al. (239) described 45 ureolytic strains of *E. coli* isolated from clinical sources. Upon reculture of stored isolates, six of these isolates generated urease-negative segregants. One of five of these strains was able to transfer conjugally the urease trait to a recipient *E. coli* via an 80- to 90-megadalton plasmid. No explanation for reversion of the remaining isolates was found at that time, but in retrospect it may have been due to molecular rearrangement of chromosomal DNA encoding urease (38; see below for further discussion).

TABLE 4. Molecular cloning of urease gene sequences

Organism	Smallest restriction fragment to encode active urease (kb)	Plasmid-encoded polypeptides		Genes localized to:		Reference(s)
		Estimated size (kilodaltons)	Method[a]	DNA length (kb)	Method[b]	
Bacillus pasteurii	*Hin*dIII, 11	ND[c]		11	S	108
Escherichia coli	*Hin*dIII, 9.4	27, 67	Maxi	3.2–5.7	D, S, T	38
Klebsiella aerogenes	*Sau*3A partial, 3.5	ND		3.5	D, S	Mulrooney and Hausinger, submitted
Klebsiella pneumoniae	*Hin*dIII-*Eco*RI, 12.3	14, 24, 25, 34, 43, 64	Mini	12.3	D, S	63
Morganella morganii	*Eco*RI-*Sal*I, 6.9	ND		4.2–5.4	S, T	Mobley, unpublished
Proteus mirabilis	*Nhe*I-*Sau*3A/*Bam*HI, 6.5	5, 2, 7.5, 18.5, 22.5, 25, 28, 68	IVTT	6.5	D, S, T	240
	*Eco*RI, 18.5	8, 10, 21, 73	IVTT, mini	4.0–5.4	B, D, S, T	98
Providencia stuartii	*Ava*I-*Pst*I, 8.5	9, 10, 25, 73	IVTT, mini	4.4–6.0	S, T, D	158, 163

[a] Maxi, Maxicell analysis; mini, minicell analysis; IVTT, in vitro transcription-translation.
[b] B, *Bal*31 mutagenesis; D, deletion analysis; S, subcloning; T, transposon mutagenesis.
[c] ND, Not determined.

Streptococcus faecium. The only other species in which a plasmid-encoded urease has been suggested was described for a strain of *S. faecium* isolated from a sheep rumen (40). Procedures consistent with curing (passage at low temperature; growth in 0.002% sodium dodecyl sulfate or 25 μM ethidium bromide) resulted in loss of urease activity. However, no plasmid DNA was demonstrated in the precured isolate. In view of this, possible explanations for the loss of urease activity may be stepwise repression of the genes on nitrogen-rich media (refer to the subsection, "Urease Regulation") or chromosomal rearrangement with gene reorganization as reported for *E. coli* (38).

Cloning of Urease Genes

Molecular cloning of urease gene sequences has been accomplished from a number of species (Table 4). With the exception of *Providencia stuartii*, in which urease was plasmid encoded, urease genes were cloned from chromosomal DNA by cosmid gene bank preparation or other methods. Clones were selected on appropriate antibiotic media and screened on various types of indicator plates.

Cloning methods. Several cloning strategies have been adopted for expression of urease genes in *E. coli*. Isolation of genes from *Providencia stuartii* was accomplished by shotgun cloning of an *Eco*RI digest of an 80-kilobase (kb) conjugative plasmid into pBR322. The genes of *Proteus mirabilis* (98, 240), *E. coli* (38), *K. aerogenes* (S. B. Mulrooney and R. P. Hausinger, submitted for publication) and *Morganella morganii* (W. Nichols, G.-F. Gerlach, and S. Clegg, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, D225, p. 91; E. Nicholson, B. D. Jones, and H. L. T. Mobley, unpublished data) were cloned by ligating *Sau*3A partial digests of chromosomal DNA into the *Bam*HI site of various cosmid vectors and packaging in vitro into lambda particles. For *Bacillus pasteurii* (108) and *Klebsiella pneumoniae* (63), chromosomal DNA was size fractionated, ligated into positive selection vectors, and used to directly transform *E. coli*.

Urease test media. For all species, cosmid or recombinant clones were selected for appropriate antibiotic resistance and screened on urease-indicating media. Commercially available Christensen urea agar (36) has been used, but it has some limitations. Since this medium is relatively unbuffered, urease-positive colonies can be identified quickly, but diffusion of ammonia rapidly turns the entire plate red, making it unsuitable for overnight incubation. This problem has been overcome by using agar supplemented with glucose (for increased acid production during fermentation) and additional peptone, yeast extract, and phosphate (for increased buffering capacity), which limits the size of the red zones around the colonies. This modified medium, termed urea segregation agar, was developed by Farmer et al. (49) for *Providencia rettgeri*. Jones and Mobley (98) reduced the urea concentration of urea segregation agar from 2 to 0.1% to accommodate the sensitivity to elevated pH of *E. coli* HB101 harboring urease-encoding plasmids. Because urea segregation agar is rich in nitrogenous compounds, this medium may be unsuitable for organisms in which urease is repressed by ammonia. To overcome this problem, Mulrooney and Hausinger (submitted) used ammonium-free M9 medium (140) adjusted to pH 6.8 and supplemented with 10% (vol/vol) LB broth, urea, and phenol red to select for clones encoding *K. aerogenes* urease. Other screening media include urea R broth (108) used for screening *Bacillus pasteurii* and urea-glucose-eosin Y-methylene blue agar (38) for identification of *E. coli* urease clones. Further screening or direct selection has been accomplished (38) by using urea as the sole nitrogen source in MOPS (morpholinepropanesulfonic acid)-urea medium, a modification (69) of the MOPS medium described by Neidhardt et al. (168).

Minimal DNA needed to encode urease. Subcloning of large DNA fragments encoding urease has been accomplished for several species (Table 4) and reveals that a fairly significant segment of DNA is necessary to synthesize an active enzyme. Transposon analysis has revealed minimum and maximum sizes for functional gene sequences to be 3.2 to 5.8 kb for *E. coli* (38), 4.4 to 6.0 kb for *Providencia stuartii* (158, 163), 4.0 to 5.4 kb or about 6.5 kb for *Proteus mirabilis* (98, 240), and 4.2 to 5.4 kb for *Morganella morganii* (Mobley, unpublished data). As discussed further below, the large span of DNA is required not only to encode the structural polypeptides that comprise the native enzyme, but also additional proteins required for assembly, nickel transport, or other functions.

DNA homology of urease genes. Although many bacterial ureases share similarities in subunit structure, there appears to be some divergence in genetic relatedness as revealed by DNA hybridization. Thus far, there appear to be at least four hybridization groups based on DNA hybridization of whole-

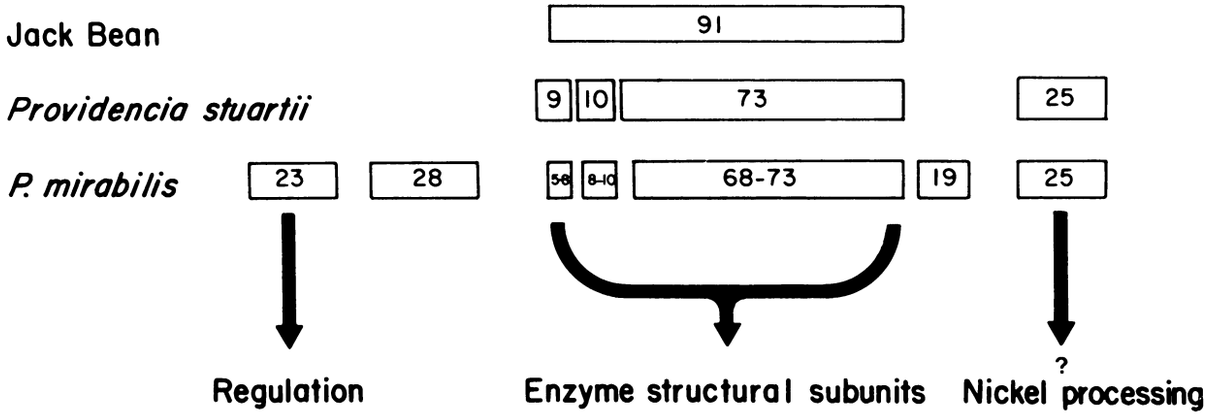

FIG. 4. Genetic organization of the urease operon. Shown are relative positions of genes encoding polypeptides associated with the urease operon of *Proteus mirabilis* (data derived from references 98 and 240), as well as those so far identified for *Providencia stuartii* (data derived from references 158 and 163). Gene products (rectangles) identified by minicell analysis and in vitro transcription-translation are labeled with apparent molecular sizes in kilodaltons. A representation of the single jack bean urease subunit is also given for comparison (molecular size from reference 224). Polypeptides that may play a role in regulation (98), that comprise the enzyme itself (163), or that may be involved in nickel transport or processing (Hausinger and Mobley, unpublished data) are identified at the bottom.

cell DNA from a limited number of species with specific urease probes from *Providencia stuartii* (97), *K. pneumoniae* (63), *Proteus mirabilis* (B. D. Jones and H. L. T. Mobley, unpublished data), and *Morganella morganii* (Mobley, unpublished data). *Providencia* and *Proteus* spp. represent group I; *Klebsiella* spp. represent group II; and *Morganella* spp. is distinct and forms group III. *C. pylori* has been placed in a fourth group by exclusion, reacting with none of the probes. Since limited work has been done in this field, only a few gram-negative species can be classified. Nucleotide sequencing efforts are in progress and will provide detailed characterization of urease gene homologies among selected bacteria.

Regulation of cloned urease genes. Expression of cloned urease sequences appears to depend on adjacent DNA sequences, growth conditions, and host cell species. Inducibility of the cloned *Proteus mirabilis* urease operon depends on the presence of specific gene sequences (98). A series of *Bal*31 deletion mutants of increasing size produced four phenotypes of urease expression: (i) basal amount of enzyme activity (not inducible); (ii) constitutive synthesis; (iii) inducible synthesis (increases 16-fold in presence of urea); and (iv) "hyperinducible" (produced a basal level, but was reproducibly induced 1,000-fold to a level similar to that of the induced wild-type organism). These data were consistent with the presence of a repressor that controls expression in a fashion similar to that of the *lac* operon. Recombinant *Providencia stuartii* urease was also found to be inducible by urea in *E. coli* (163).

The cloned sequences of *K. aerogenes* (Mulrooney and Hausinger, submitted) appear to be regulated by the nitrogen regulation system as high levels of expression were demonstrated in nitrogen-limited medium. Also, excellent expression (>100-fold of that of the wild type) was observed not only for the wild-type *K. aerogenes* host transformed with recombinant urease-encoding plasmids, but also in the heterologous hosts *Salmonella typhimurium* and *E. coli*.

Bacterial Urease Operon

Delineation of the number and organization of cloned urease genes has been accomplished by subcloning *Sau*3A partial digests (Mulrooney and Hausinger, submitted), deletion analysis (38, 63, 98, 158, 240), *Bal*31 digestion (98), and transposon mutagenesis (38, 98, 158, 163, 240). Inactivation of urease is easily assayed, and therefore large numbers of mutants can be screened quickly. From a consensus of cloned sequences, it appears that about 6 kb of DNA is necessary for urease activity (Table 4), with the exception of *K. aerogenes* which appears to require less DNA for activity.

Expression of polypeptides. Polypeptides encoded by cloned urease sequences have been identified by radiolabeling plasmid-encoded peptides in vivo in *E. coli* maxicells (38) or minicells (63, 98, 158), by in vitro transcription-translation of purified DNA (98, 163, 240), or by purification of the recombinant enzyme (163; Mulrooney and Hausinger, submitted). As evident in Table 4, the cloned genes encode more polypeptides than those that comprise the three structural protein subunits of urease (see subsection, "Urease Structural Properties"). However, owing to their localization and their requirement in ureolysis, these polypeptides are thought to be part of the urease operon.

Gene organization. The genetic organization of bacterial urease operons is similar in those organisms that have been the best studied, namely, *Proteus mirabilis* (98, 240) and *Providencia stuartii* (158, 163). The organization of the urease operon is schematically illustrated in Fig. 4. In both species, the urease structural genes are contiguous and are transcribed as polycistronic messenger RNAs from smallest to largest polypeptides (5 to 9, then 8 to 10, and then 68 to 73 kilodaltons). Preceding the urease structural genes from these two species are regulatory genes necessary for induction by urea. Following the structural genes and apparently not transcribed on the same messenger RNA are at least two accessory genes (encoding proteins of 19 and 25 kilodaltons) for which no functions have been assigned. Postulated roles for these proteins include involvement in urea transport, nickel transport, or nickel processing. Although somewhat less well characterized, the *K. pneumoniae* urease operon also is complex, encoding at least six polypeptides (63).

Although the number of genes and the gene organization in the *K. aerogenes* urease operon have not been determined, it is appropriate to comment here on a partial protein sequence which was described earlier (Fig. 3). The N-

terminal 19 amino acids in the sequence of the 72-kilodalton subunit from *K. aerogenes* urease matched 10 of 19 amino acids in an internal sequence of the jack bean enzyme, beginning at residue 272. If the urease gene organization of *K. aerogenes* is similar to that of *Proteus mirabilis* or *Providencia stuartii*, then the gene encoding the large subunit is immediately preceded by the genes for the two small subunits. This organization may suggest that the single subunit jack bean enzyme is directly homologous to the three subunits in bacteria encoded by a single RNA transcript (also see Fig. 4). From preliminary sequencing data (Jones and Mobley, unpublished data), the predicted amino acid sequence of the N terminus of the large-subunit polypeptide of *Proteus mirabilis* urease shows strong similarity to the *K. aerogenes* sequence (15 of 18 amino acids are identical) and some similarity to the jack bean sequence (10 of 19 amino acids are identical). Further sequence analysis will determine the extent of similarity between the plant and microbial enzymes.

Urease-positive *E. coli* represent <1% of all *E. coli* clinical isolates (239), and the urease-positive phenotype appears to be unstable. Instability may occur by either loss of a plasmid-encoded trait or specific genomic rearrangement within the urease operon (38). This loss of activity was irreversible, in contrast to phase variation phenomena.

Eucaryotic Ureases

The only molecular biological studies of urease from microbial eucaryotes are genetic analyses as described below. For comparison with microbial urease studies, the results of molecular biological characterization for plant ureases are also summarized.

Fungal urease genetics. Four complementation groups have been identified that are necessary for *Aspergillus nidulans* urease activity (129, 176). *ureA* is the structural gene for the urea transport protein. *ureB* encodes the single subunit urease enzyme. *ureC* encodes a product necessary for enzyme activity but the function is unknown. *ureD* is suspected to be necessary for synthesis or incorporation of nickel cofactor. A mutation in *ureD* can be overcome by growth in the presence of 0.1 mM $NiSO_4$ (128).

In *Ustilago violacea*, two complementing urease loci have been identified (4). *ure-1* encodes the single-subunit enzyme. *ure-2* represents a urea permease gene, as mutants in this locus demonstrate no activity on phenol red urea test agar, whereas lysates show wild-type enzyme activity.

For *Neurospora crassa*, Kolmark (115) and Buremalm and Kolmark (28) isolated two urease-deficient mutants and determined by crossing and ascospore isolation that the markers could complement one another and were physically separated by the *am* gene. Haysman and Howe (82) and Benson and Howe (10) presented evidence that four complementing loci are involved in production of urease. The authors suggested that each locus encodes a structural function as opposed to regulatory elements. That is, we can speculate that complementation groups may encode urease structural subunits, a urea transport system, nickel transport protein, or other functions.

Plant urease molecular biology. Genetically, the best-characterized eucaryotic urease is that of soybean (*Glycine max*). This plant contains two urease isozymes whose expression is regulated in a tissue-specific and temporal manner (85). Mutational analysis has shown that the ubiquitous and embryo-specific structural genes are located at distinct loci. In addition, pleiotropic mutants deficient in both ureases have provided evidence for two other loci which are involved in urease maturation (154). The defect in processing may involve, for example, the inability to correctly incorporate nickel ion into the urease apoenzymes.

A portion of the soybean embryo-specific urease gene has been cloned. Krueger et al. (121) used a synthetic base pair mixed oligonucleotide based on a seven-residue, amino-terminal amino acid sequence from soybean urease to screen genomic libraries of soybean. Homologous clones were screened with a mixed 15-base-pair oligonucleotide based on a jack bean urease peptide (139). The subclone which hybridized was sequenced and contained a deduced amino acid sequence that matched 108 of 130 (83%) amino acids determined for jack bean by Mamiya et al. (139). The oligonucleotide probe hybridized with a 3.8-kb soybean embryonic messenger RNA present in the wild type, but not with that synthesized by a urease-negative mutant (121).

CONCLUSIONS

Urea degradation by microorganisms plays an important part in several disease states, in ruminal and gastrointestinal microecology, and in environmental nitrogen cycling. The seemingly simple hydrolysis reaction belies a surprisingly complex biological process. Catalysis is carried out by a large, multicomponent, nickel-containing enzyme. The urease subunits are encoded on a urease operon which also includes several other genes. The roles for these ancillary genes are likely to include transport of urea and nickel, as well as proteins involved in nickel processing.

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